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SECT. II.—NATURAL HISTORY.

THE MICROSCOPE,

AND ITS APPLICATION

TO

VEGETABLE ANATOMY AND PHYSIOLOGY.

THE MICROSCOPE,

AND

ITS APPLICATION

TO

VEGETABLE ANATOMY AND PHYSIOLOGY.

BY

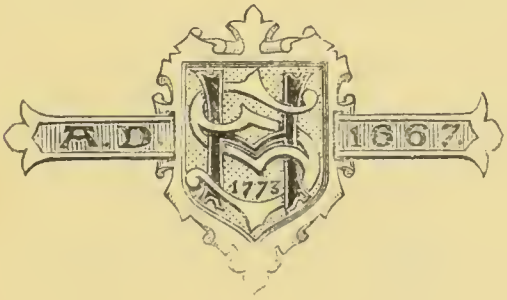
DR. HERMANN SCHACHT.

EDITED BY

FREDERICK CURREY, M.A.

SECOND EDITION.

Considerably Enlarged, with Numerous Illustrations.



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TRANSLATOR'S PREFACE

TO THE

SECOND EDITION.

THE rapid sale of the First Edition of this Translation has rendered a Second necessary; and I wish to direct attention very briefly to the points in which the present Edition differs from the preceding. Four chapters have been added at the beginning of the book; the first of which relates to some elementary principles of Optics essential to a proper comprehension of the Microscope; the second contains a description of different kinds of English Microscopes, including, as far as is necessary, the details of their different parts; the third contains an account of the accessory apparatus and chemical reagents necessary for microscopical investigations in botany, and the fourth relates to the preservation of specimens. I have myself added these Chapters with the view of rendering the work more complete as a Manual for English Students, and am, therefore, responsible for the contents of them, with the exception of the list of chemical reagents in Chapter III., which is to be found in the original work. Besides the ad-

dition of the above four Chapters, I have been furnished by Dr. Schacht with a quantity of new matter in manuscript, being the result of his investigations since 1852, and this new matter has been incorporated in the text; the present Edition is, therefore, considerably in advance of the original work. At the suggestion of Dr. Schacht, I have added the Chapters IX, X, and XI, which contain an interesting account of the embryogeny of the Coniferæ, and are a translation of a portion of a work published by him in the course of last summer, entitled "Beiträge zur Anatomie und Physiologie der Gewächse." Chapters VII and VIII of the first Edition, which related to drawing and to the preservation of objects, have been omitted; but the student will find what is necessary upon these points under the head of "Delineating Apparatus," in Chapter III, and in Chapter IV, which treats of the preservation of specimens.

FREDERICK CURREY.

Blackheath.

December 19th, 1854.

TRANSLATOR'S PREFACE

TO THE

FIRST EDITION.

THE Work of Dr. Schacht, of which a Translation is now offered to the public, relates to a branch of Microscopical Science which has not hitherto formed the subject of a separate Treatise; and the high reputation of the Author, and the interesting nature of the subject, have induced a belief that the present Version is likely to meet with a favourable reception.

It has been thought advisable to omit the greater part of the description of foreign Microscopes and auxiliary instruments contained in the Original Work. These details would, for obvious reasons, be uninteresting, if not useless, to the English reader. There is no doubt of the superiority of English instruments over those described by Dr. Schacht; and the elaborate and able Treatise of Professor Quekett affords all the necessary information upon the subject of English Microscopes, &c.

The high price of good English Microscopes has hitherto been an impediment to the progress of Microscopy,

and much attention has lately been directed to the production of cheaper instruments. A very useful and convenient form of Student's Microscope is represented in the Frontispiece, which has been designed by Mr. Samuel Highley, Jun., of Fleet Street, and may be had at a very moderate price.

The figures of the Original Work, and their descriptions, have been incorporated into the text of the Translation, by which means the inconvenience of constant reference to the plates and their explanation is avoided. The figures of the foreign instruments, and a few other figures, which were not essential for the elucidation of the subject, and which would have increased the expense of the Translation, have been omitted.

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THE MICROSCOPE,

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VEGETABLE ANATOMY AND PHYSIOLOGY.

CHAPTER I.

PRELIMINARY IDEAS UPON OPTICS.

Before entering upon the description of the instruments and apparatus necessary for the conduct of microscopical investigations, it may be interesting and useful to bring to the student's notice a few of the leading preliminary facts connected with optical science, in order that when he enters upon the use of the microscope he may have some idea of the *causes* of the effects produced, and may not be in the position of merely seeing and admiring them.

Light is the agent by which the objects of the material world become visible to our senses. Upon the nature of the constitution of light, different opinions have been entertained. Sir Isaac Newton and his followers supposed that light consisted of minute, material particles, darted out in straight lines or rays from luminous bodies, and that these particles, by impinging upon the eye, produced the sensation of vision. According to the theory of modern philosophy, the sensation of light is produced by the vibrations of the particles of a subtle elastic medium supposed to pervade all space, and to which the name *Ether* has been applied.

It will not be necessary for our purposes to go into the question of these two theories, beyond remarking that the latter is the one which is generally admitted at the present day, although many optical phenomena are capable of explanation according to either the one or the other.

Laws of Light.—The three principal laws of light are those of reflexion, refraction, and the chromatic dispersion of light. Upon each of these we shall have to say a few words, but will first explain some terms of constant occurrence, and which it is necessary should be understood.

A *ray* is the smallest portion of light which we are capable of conceiving, and is represented by a line drawn in the direction in which the light is supposed to proceed. A small assemblage of rays constitutes a *pencil* of light, which may be either conical, as when the rays diverge from a point (fig. 1), or cylindrical, when consisting of parallel rays (fig. 2).

Fig. 1.

Fig. 2.

An assemblage of rays, if too large to come under the denomination of a pencil, is called a beam. When rays diverge from a point or converge to a point, the point from which they diverge or converge is called the *focus*. The *principal focus* is the point to which parallel rays converge after reflection or refraction.

The normal to a surface at any point is a straight line perpendicular to the surface at that point.

When a ray of light is incident upon a plane surface, the angle which the direction of the ray makes with the normal to the surface at the point of incidence, is called the *angle of incidence;* and when the incident ray is reflected or refracted by the surface, the angles which the reflected and refracted rays respectively make with the normal, are called the *angles of reflexion* and *refraction.*

Bodies which do not admit of the passage of light through them are called *opaque,* whilst those through which light passes are called *transparent.*

There are no bodies in Nature which are either perfectly opaque, or perfectly transparent. Bodies composed of the densest material, such as gold, when sufficiently reduced in

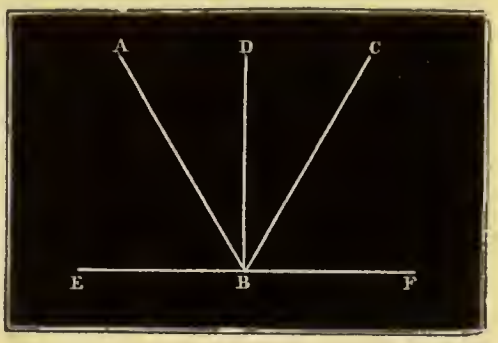
thickness, admit of the passage of a small portion of light, and bodies apparently of the greatest transparency always intercept some portion of light, however small.

Reflexion.—When light is incident upon an opaque body, it is reflected, or thrown back, into the medium in which it is propagated, and this reflexion takes place in different ways, according to the nature of the surface upon which the light is incident. When the surface is rough and unpolished, the light is reflected in all directions from every point of the surface, each point becoming, as it were, the origin of a pencil of rays, which diverge in all directions. This mode of reflexion is called *irregular reflexion*, and by means of it the outlines and forms of bodies in general are rendered visible.

When light is reflected from a polished surface, the reflexion takes place according to a certain law, which may be thus expressed, viz. :—

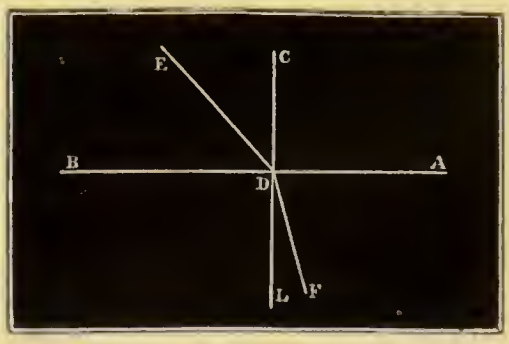
The incident and reflected rays lie on opposite sides of the normal to the surface at the point of incidence, and the angle of incidence is equal to the angle of reflexion.

Fig. 3.



Suppose E B F in fig. 3 to be the reflecting surface, A B a ray of light incident upon it at B, and let B D be perpendicular to E B F ; then if the angle D B C be taken equal to the angle A B D, B C will be the course of the ray A B after reflexion.

Fig. 4.



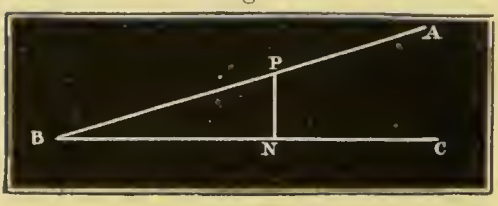
Refraction.— When a ray of light passes obliquely from one transparent medium into another of different density, it is bent out of its course, or, as it is called, *refracted.*

Thus, let E D (fig. 4) be a ray of light incident upon A B,

which we may suppose to be the surface of water, then the ray E D will be turned out of its course, and will proceed in the direction D F. In this case, the density of water being greater than that of air, the refraction takes place *towards* the perpendicular (D L) to the surface. When the density of the medium *into* which the refracted ray passes is less than that *out of* which it passes, the refraction is *from* the perpendicular.

Refraction takes place according to a certain law, and in order to render that law intelligible, it is necessary in the first place to explain what is meant by the sine of an angle.

Fig. 5.



If A B C (fig. 5) be any angle, and if from any point P in B A, a line P N be drawn perpendicular to B C, then the ratio of the line P N to the line P B, or $\frac{P N}{P B}$, is called the sine of the angle A B C, or as it is expressed mathematically, $\frac{P N}{B P} = \text{sin. B.}$

The law of refraction is as follows :—

The incident and refracted rays lie in the same plane with, but on opposite sides of, the normal to the surface at the point of incidence, and the sine of the angle of incidence bears a certain ratio to the sine of the angle of refraction, which ratio depends upon the nature of the media between which the refraction takes place.

If the angle of incidence be represented by ϕ, and the angle of refraction by ϕ', this law may be shortly expressed thus

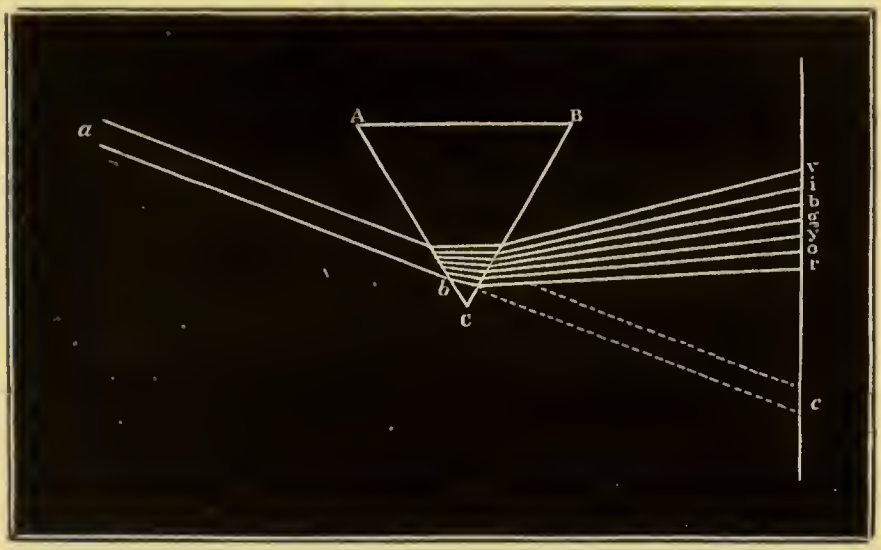
$$\text{sin. } \phi = \mu \text{ sin. } \phi'$$

where μ is constant.

Chromatic Dispersion of Light.—If a beam of solar light *a b c* (fig. 6), be admitted through a round hole in the window-shutter of a darkened room, and received upon a screen, a circular spot of white light will be visible upon the screen at the point where the beam of light impinges upon it. If, now, a glass prism, A C B, be placed so as to intercept the course of the light, in the manner represented in the figure, the beam will

be decomposed; the light will be refracted upwards, and an oblong image containing the colours, popularly known as the prismatic colours, will be visible upon the screen, instead of the spot of white light. These prismatic colours are seven in num-

Fig. 6.



ber, and succeed one another in the following order; viz., violet, indigo, blue, green, yellow, orange and red. The assemblage of them is called the solar spectrum, and the cause of the appearance has been ascertained to be the different refrangibility of the different rays of light which enter into the composition of a beam of ordinary sunlight. The violet rays being more easily refracted than any others, are diverted from their course to a greater extent than the indigo, the indigo to a greater extent than the blue, and so on, until we arrive at the red rays which undergo the least refraction of all.

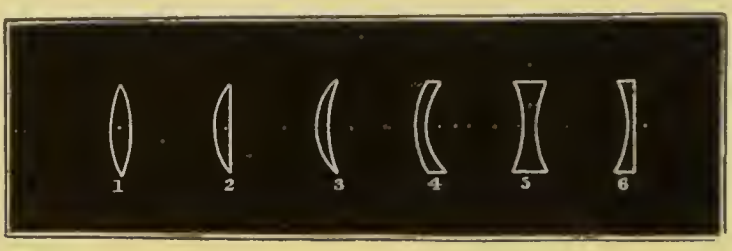
Lenses.—A lens is a portion of a transparent medium bounded by spherical surfaces. This definition would include a lens with one-side plane, such as a plano-convex lens, inasmuch as the plane side may be considered to be a spherical surface with an infinite radius.

For optical purposes glass is the material always employed in the manufacture of lenses. Although lenses might be made of

any transparent material, and with surfaces other than spherical, no material has yet been met with which combines so many advantages as glass; and this fact, together with the difficulty of grinding accurately any other than a spherical surface, has led to the universal adoption for microscopical purposes of glass lenses with spherical surfaces.

In fig. 7 are represented the principal forms of lenses, and the names attached to these different forms are as follows :—

Fig. 7.



1. Double convex; 2. Plano-convex; 3. Meniscus; 4. Concavo-convex; 5. Double concave; 6. Plano-concave.

It will be observed, that in the meniscus, the radius of the convex surface is less than that of the concave surface; whilst in the concavo-convex the contrary is the case, that is, the radius of the concave surface is less than that of the convex.

Nos. 1, 2, and 3 are called convergent lenses, because a pencil of parallel rays incident upon them is rendered convergent.

Nos. 4, 5, and 6 render a pencil of parallel rays incident upon them divergent, and are therefore called divergent lenses.

Whatever be the form of a convergent lens, a double convex lens with equal radii can always be found, which will produce the same optical effect; and, on the other hand, a double concave lens, with equal radii can always be found, which shall be optically equivalent to any form of divergent lens.

Formation of Images by Lenses.—A full investigation of the manner in which images are formed by lenses would occupy more space than can be afforded in a work like the present, and would, moreover, involve a discussion of mathematical formulæ, which would be out of place. The following rules, however, applicable to two particular cases of the formation of images, and

which will occur in the discussion of the construction of microscopes, may here be stated.

1. When an object is placed before a double convex lens, at a distance from the lens *less* than that of its principal focus, a virtual erect and magnified image of the object is formed on the same side of the lens as the object, and at a distance from the lens greater than that of the object.*

2. When an object is placed before a double convex lens, at a distance from the lens *greater* than that of the principal focus, but *less* than twice the focal length of the lens, a real, inverted, magnified image of the object is formed on the opposite side of the lens.

Fig. 8.

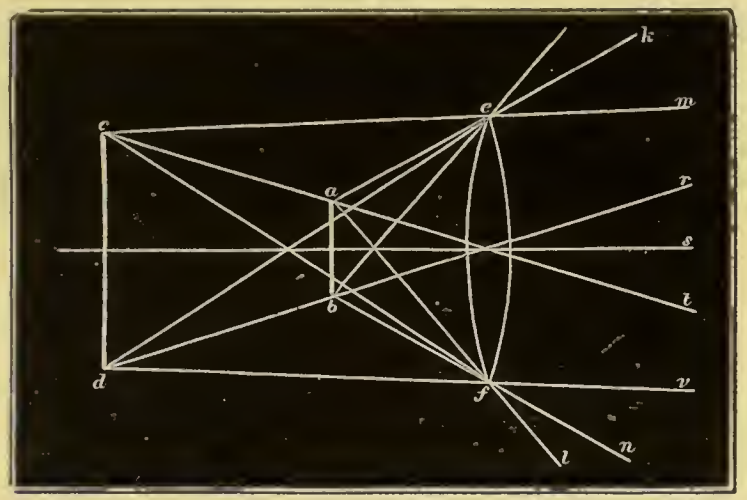
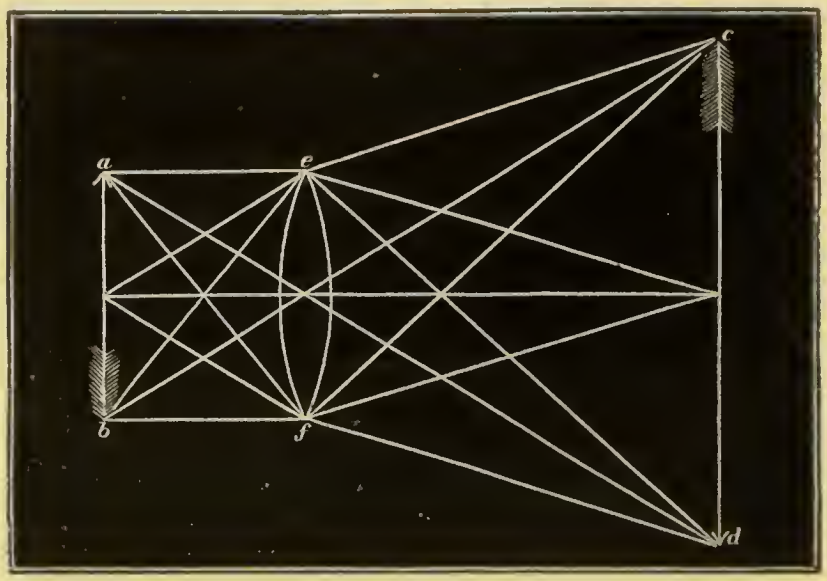


Fig. 8 will illustrate rule 1. Let *a b* be an object placed within the principal focus of the double convex lens *e f*, and let *e a f*, *e b f* be the pencils of light from the extreme points of the object; the rays *a e*, *a f*, (which are the extreme rays of the pencil *e a f*) will be refracted by the lens into the directions *e m*, *f n*, and after refraction will apparently diverge from the point *c*. In like manner the rays *b e*, *b f* will be refracted so as to diverge apparently from the point *d*, and thus a virtual, erect, and magnified image of the object *a b* is formed at *c d*.

* A *virtual* image, is one in which the rays of light do not actually pass through the points of the image, as is the case when the image is *real*.

Fig. 9 will illustrate rule 2. If *a b* be an objeet plaeed a little beyond the principal focus of the lens *e f*, the peneil *e a f*, diverging from the extremity *a* of the objeet, will, after refrae-

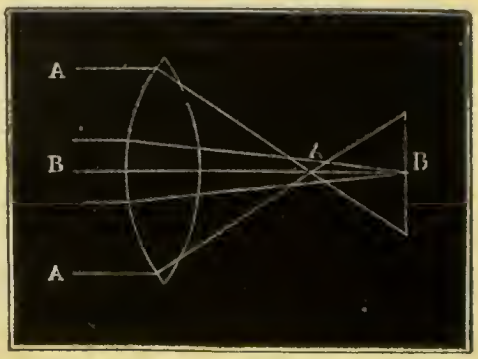
Fig. 9.



tion, be brought to a focus at a point *d* on the other side of the lens, and in like manner the pencil *e b f* will be refracted to a foeus at *c*, and thus a real inverted magnified image of the objeet will be formed at *c d*.

In the formation of these images we have supposed all the rays of each peneil to be brought to an exact focus by the lens. This, however, is never the ease with an ordinary lens, on aeeount of the fact, that the rays whieh fall upon a lens near the margin are more strongly refracted than those whieh pass through nearer to the centre; henee arises an aberration known by the name of spherieal aberration, whieh fig. 10 will serve to explain. A peneil of light (here supposed to consist of parallel rays) falls upon the lens; the rays A near the margin are refracted to the foeus A, whilst the rays

Fig. 10.



B near the centre are refracted to B. It will at once be seen that the effect of this aberration will be to cause an indistinctness in the image. This indistinctness may be remedied by limiting the aperture of the lens, which may be done by covering up either the margin or the centre of the lens; in the former case, the image is formed by the central rays alone; in the latter, by the peripheral rays alone. The distance, A B, is called the *longitudinal* spherical aberration; and the line through B at right angles to B B, and which is the diameter of the circle of aberration over which the rays are spread, is called the *lateral* aberration.

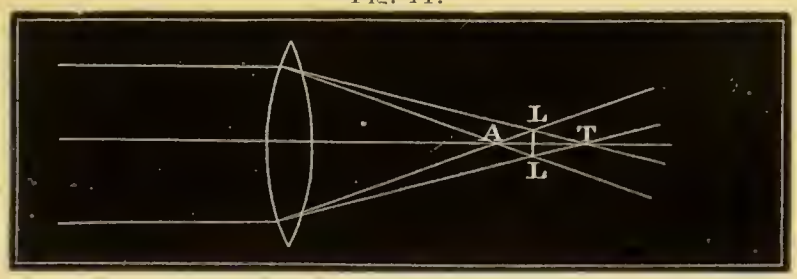
The amount of spherical aberration varies according to the shape of the lens, and the manner in which it is presented to the light. In a plano-convex lens with its plane side turned to parallel rays, the spherical aberration is $4\frac{1}{2}$ times the thickness of the lens; whilst, if its convex side be turned to parallel rays, the aberration is only $1-\frac{17}{100}$th.

It has been ascertained that the lens which has the least spherical aberration is a double convex, whose radii are in the proportion of 1 to 6.

Spherical aberration might be entirely got rid of if it were possible to construct a lens the section of which should be an ellipse or hyperbola, but practical difficulties have prevented the manufacture of such lenses, so that in order to get rid of spherical aberration, it is necessary that two or more lenses should be combined in such a manner as to make opposite aberrations correct each other. We shall have to refer again to this subject in speaking of Dr. Wollaston's doublet.

Chromatic Aberration.—Another cause of confusion in the

Fig. 11.

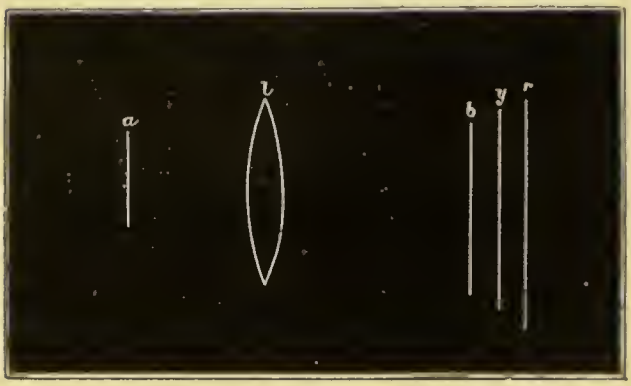


formation of images arises from what is called chromatic aber-

ration. This aberration is produced by the unequal refrangibility of the differently-coloured rays, which together constitute white light. In fig. 11 the two rays of white light which fall upon the margin of the lens are decomposed and refracted, the violet portions of the ray being refracted to A, and the red portions to T; the other coloured portions being refracted to points intermediate between A and T.

The effect of this aberration is to cause the images of objects to be fringed with colour at the margin, the cause of which is thus explained. If (*a*) fig. 12, be an object placed before a double convex lens (*l*), at a distance a little beyond the principal focus, an inverted magnified image will be formed on the other side; but owing to the different refrangibility of the different rays of light, the red image (*r*) will be formed at a distance from the lens greater than that of the yellow image *y*, and the yellow image (*y*) at a greater distance than the blue image (*b*). Now, if we imagine a screen, or the retina of the human eye, to be placed so as to receive the image (*r*), such image (setting aside for the present the spherical aberration) would be a distinct red image of the object (*a*), if it were not for the existence of the yellow and blue images; but the blue and yellow pencils after being brought to a focus at (*b*) and (*y*) respectively, diverge from the points of those images, and fall upon the red image (*r*). A portion of the divergent yellow and blue pencils mingle with the red image and produce white light; but the rest of the yellow and blue pencils, owing to their divergence, overlap the red image, and the result is, that the image formed by all the rays together is white only in the middle, and fringed with colour at the edges.

Fig. 12.



By the use of a compound lens formed of glass, having dif-

ferent refractive and dispersive powers, the aberration is so far diminished that achromatism is practically produced.

The difference of the deviations of any two rays of the spectrum divided by the mean deviation, is called the *dispersive power* of the medium for those rays.

CHAPTER II.

THE APPLICATION OF THE PRINCIPLES EXPLAINED IN CHAPTER I. TO THE MICROSCOPE.

We will now proceed to examine how the principles explained in the preceding chapter are applicable to the microscope. This instrument, as its name implies, is constructed for enabling the eye to obtain distinct perception of objects, which, from their minuteness, would be invisible, or only indistinctly visible, to unaided vision. The eye has the power, within certain limits, of adapting itself, that is, of altering its refractive power, in such a manner as to obtain distinct vision either of distant or of near objects; but when an object is placed within a particular distance from the eye, the pencils of light proceeding from the object become so divergent, that the eye is unable to bring them to a focus on the retina. This distance varies with different individuals, but may be taken at an average to be 10 inches. If, now, a double convex lens be placed between the eye and the object, the pencils of light being refracted by the lens, are made to emerge with a degree of divergence so much reduced, as to enable the eye to bring them to a focus. Fig. 8 shews the course of the pencils of light, and the manner in which the divergence is reduced; and the result is, that to an eye placed in the axis of the lens, the object *a b* appears to be situated at the distance, and to be of the magnitude of the object *c d*.

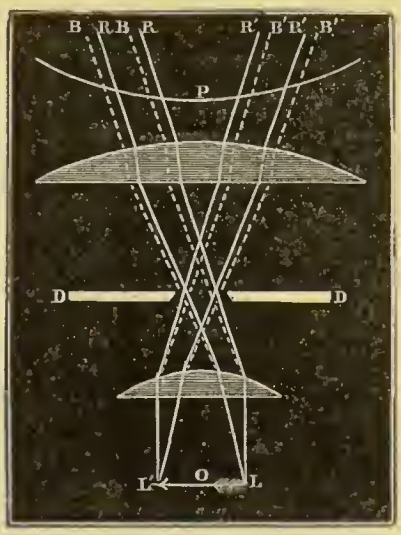
Simple Microscope.—A simple microscope in its most simple form is nothing more than a convergent lens of short focal length interposed between the eye and the object, by which the observer is enabled to obtain distinct vision at a distance considerably less than would be practicable with the naked eye. By the use of such a lens the apparent magnitude of any object

is increased in the proportion which the limit of distinct vision, say 10 inches, bears to the short distance at which the eye is enabled to see distinctly by the aid of the lens; the term *simple microscope*, however, is in practice applied to instruments used for dissection in contradistinction to the compound microscope. Such an instrument will be described hereafter.

Doublet.—The doublet generally used is that invented by Dr. Wollaston, and consists of two plano-convex lenses placed with their convex sides towards the eye, the focal length of the lenses being in the proportion of 1 to 3. The lens of shortest focus is placed nearest to the object. The effect of this doublet is to remedy the spherical and chromatic aberration in the following manner, as explained by Mr. Ross, in the *Penny Cyclopædia*, article "Microscope."

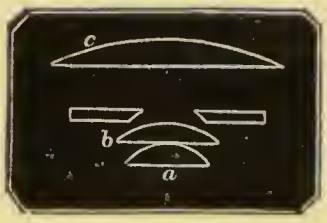
Fig. 13. P represents a portion of the pupil, D D the diaphragm or stop, and L′ O L, the object. Each of the pencils of light from the extremities of the object L′ L is rendered eccentric by the stop, and the ray that passed through the first lens near to the centre, is made to pass through the periphery of the second lens, and on the opposite side of the common axis, P O; thus, each is affected by opposite errors, which in some measure neutralise each other; and the rays R B, R B, emanating from L, being bent to the right in the lower lens, and to the left in the upper, and as the most refrangible of the coloured rays, the blue, is altered in its course at each bending, and falls near the margin of the second lens, where the refraction is more powerful than in the centre, the blue and red rays will emerge very nearly parallel, and, consequently, colourless to the eye; thus, the chromatic aberration is almost, if not entirely, destroyed, whilst the spherical has been considerably diminished by the circumstance that the pencil which passes one lens nearest the axis, passes the

Fig. 13.



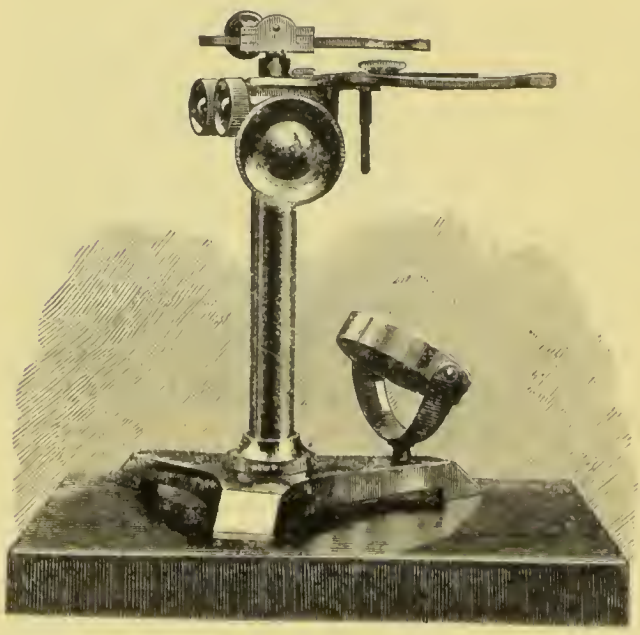
other nearest the margin; but however carefully a doublet of this form may be constructed, there must, of necessity, be some small amount of error; the central pencil will occupy the same relative position in both lenses, and the correction of this will consequently be imperfect, and all those rays intermediate between the centre and the margin will vary according to their distance from one or the other; but allowing this, the doublet is, nevertheless, vastly superior to any single lens of the same power, and may be made to transmit a pencil of an angle from 35° to 50° without any very sensible errors, and to exhibit most of the usual test-objects.

Fig. 14.



Triplet.—This instrument was invented by Mr. Holland in 1832, and consists, as will be seen by fig. 14, of two lenses placed close together, with a stop between them and the posterior lens. The first bending being effected by two lenses instead of one is accompanied by smaller aberrations, which are, therefore, more completely balanced or corrected at the second bending in the opposite direction by the third lens. This combination, although called a triplet, is, in fact, a doublet, in which the anterior lens is divided into two. It is capable of transmitting a pencil of 65°.

Fig. 15.

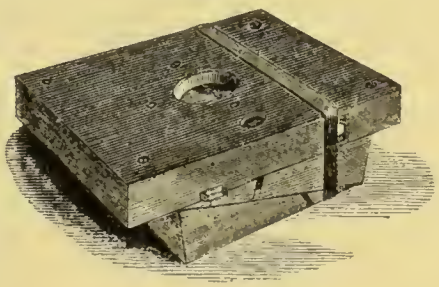


Dissecting Microscope.—There are many different sorts of dissecting microscopes, which vary according to the fancy of the makers; but as the principle of all must be the same, it will be sufficient

to refer to the accompanying fig. 15, which represents one of the best construction by Mr. Ross. The principal points to be attended to in selecting a dissecting microscope are to see that the stage is of sufficient size and strength, and that the arrangements for holding the lenses and moving them in different directions, are convenient. In the instrument in fig. 15, the arm at the top which carries the lens-holder has a forward motion by rack and pinion, and a traversing motion on a pivot, by which means the lens can be carried in any direction over the stage. The adjustment of the focus is effected by the large milled head at the side. This instrument is usually furnished with lenses of 1 inch, $\frac{1}{2}$ inch, $\frac{1}{4}$ inch, and 1-10th inch focal lengths, and sometimes with a Wollaston's doublet. The doublet may well be dispensed with, if the observer is possessed of a compound achromatic microscope. In carrying on delicate dissections with this microscope, it is advisable to make use of the arm-rests, which will be described hereafter in the chapter on accessory instruments. Mr. Ross' 1 inch achromatic object-glass may be used in dissecting with this instrument, and will be found most agreeable.

Figs. 16, 17, and 18, represent another form of dissecting microscope, called "Quekett's Dissecting Microscope," lately produced by Mr. Highley. Fig. 16 shews the instrument folded up with an Indian-rubber band round it, in a manner which admits of its being carried in the pocket. The two wedge-shaped pieces of wood underneath unfold and form the legs (*see* figs. 17 and 18). Fig. 17 shews the internal arrangement and the manner in which the mirror, lenses, and lens-holder are packed away. The straight, flat bar, on the right in fig. 17, serves to keep the legs from closing together (*see* fig. 18), and also as a support for the mirror which slides into a piece of brass tubing attached to the flat bar. The circular hole at the lower end of fig. 17 is another piece of brass

Fig. 16.



tubing, into which the lens-holder slides. The instrument is furnished with three lenses, and is to be had at a moderate price.

Fig. 17.

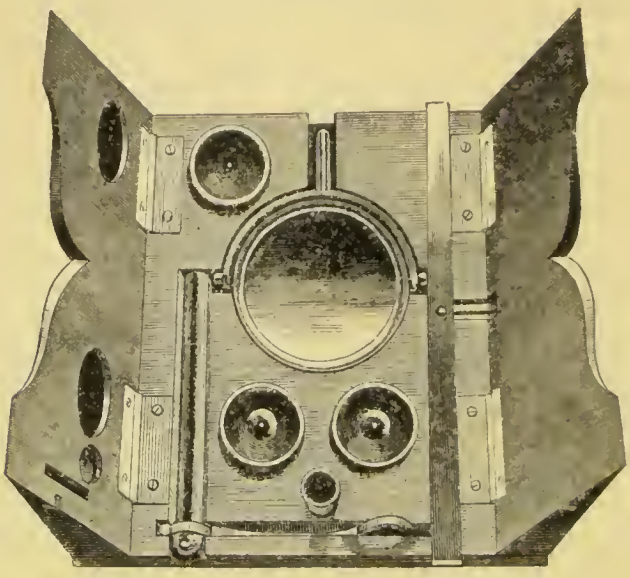
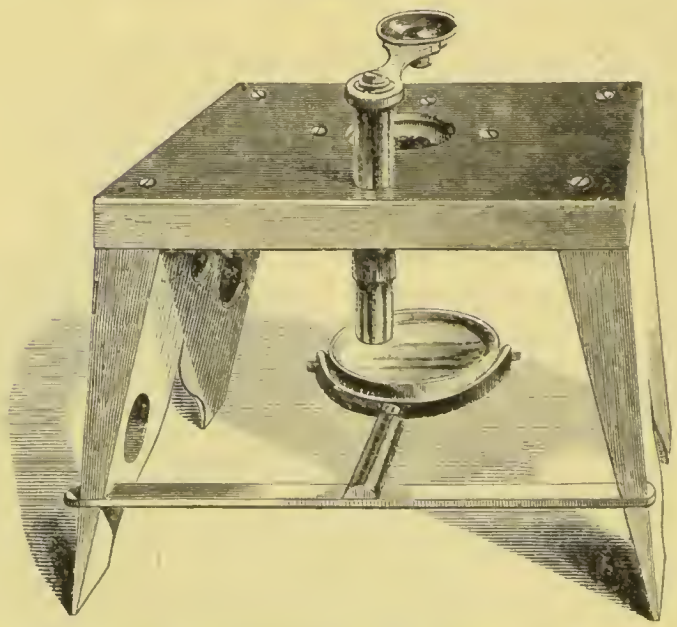


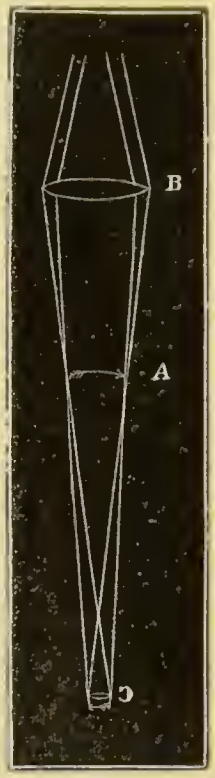
Fig. 18.



The Compound Microscope.—The action of a common compound microscope will be understood by reference to fig. 19. The object to be viewed is placed before the anterior lens, which

is called the object-glass, at a distance from the glass somewhat greater than that of its principal focus; the effect, therefore, according to the principles before explained, will be, that an inverted magnified image will be formed behind the object-glass, as at A. This image is viewed through another glass, B, called the eye-glass, by which it is further magnified. It is obvious that by this arrangement, the extent to which the image is magnified is represented by the product of the magnifying powers of the object-glass and the eye-glass. The construction represented in the figure is that of a compound microscope, in which nothing has been done to correct the errors of spherical and chromatic aberration. Such an instrument is never employed at the present day in scientific researches, but it affords an easy explanation of the *principle* of the compound microscope, which continues the same under the improved construction of later years.

Fig. 19.



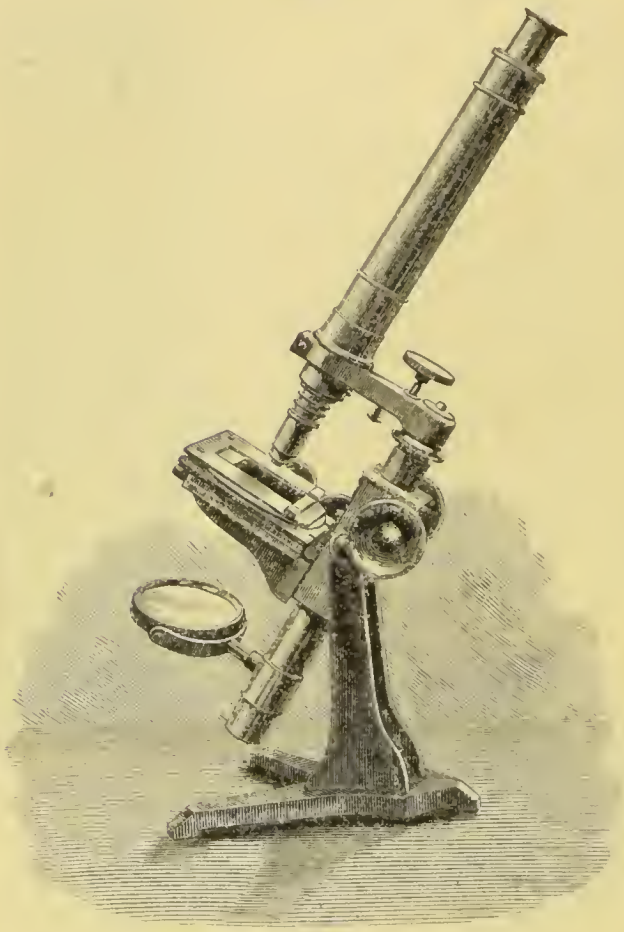
Compound Achromatic Microscope.—A general idea of a modern compound achromatic microscope will be obtained by inspection of the Frontispiece and of fig. 20. Fig. 20 represents one of Mr. Ross' instruments of the best construction, with stage movements. The Frontispiece represents a microscope designed by Mr. Highley, which is exceedingly useful and convenient, and of a less expensive kind. Fig. 21 represents Quekett's dissecting microscope, arranged as a travelling instrument, with a compound body. In describing the different parts of compound achromatic microscopes, I shall first speak of the mechanical parts, and then of the optical parts; the former include the stand, the body, the stage, and the arrangements for adjustment; and the latter, the mirror, the object-glasses, and eye-glasses.

MECHANICAL PARTS.

The Stand.—The stand of the compound microscope is usually composed of a tripod with two upright pillars. Between

these pillars is a bent bar, which works in a joint. The bar carries at one end the compound body, to which the object-glass and eye-piece are attached, and at the other end, the stage and the mirror.

Fig. 20.



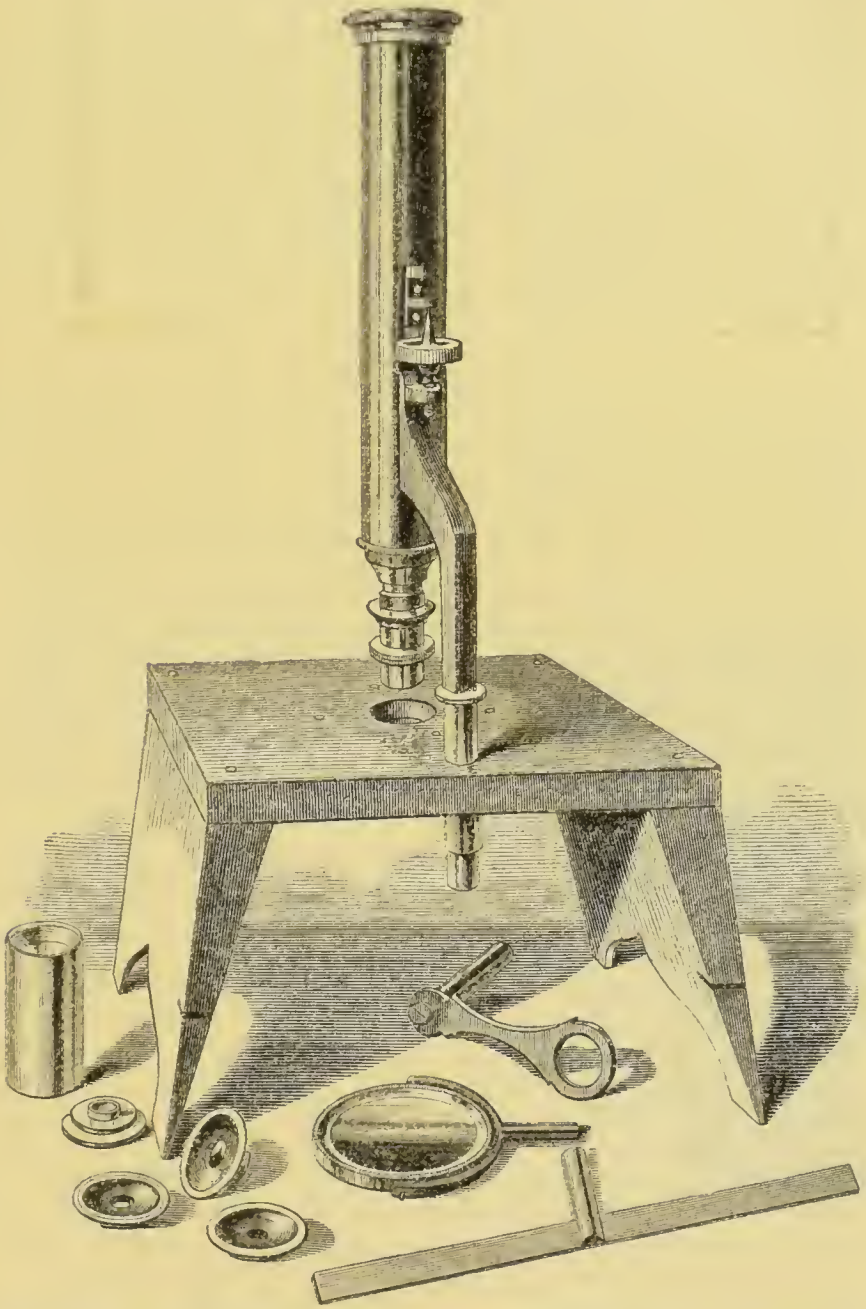
The Body. — This consists of a long brass tube, which carries the object-glass at one end and the eye-piece at the other. The object-glasses are screwed on to the body, but the eye-pieces are attached by their own tubes, which slide into the tube of the body.

The Stage. — This is the part upon which the objects to be examined are placed. It consists of a plate of brass, having another plate, called the object-plate, fitted to it, and made so as to slide up and down upon it. The object-plate has a raised ledge at the lower end for supporting the object when the instrument is inclined. In the more expensive microscopes the object-plate is made moveable in different directions by means of the screws with milled heads, partly seen in fig. 20.

The Adjustment.—The adjustment of the instrument (*i. e.*, the means by which any object on the stage is brought into the focus of the object-glass) is effected by moving the compound body. This motion is sometimes produced by sliding the body through

its holder, as will be understood by referring to the Frontispiece, but more generally by means of a rack and pinion. In the

Fig. 21.



microscope represented in fig. 20, a triangular bar is fitted into the bar which carries the stage, and this triangular bar is

moved up and down by means of the action of the large milled head seen in the figure, and of a corresponding milled head on the opposite side. In all the best microscopes there is an additional apparatus for adjustment, called the *fine adjustment*, by which the object-glass may be brought very gradually nearer or further from the object. This is indispensable when high powers are employed. It is effected by a screw with a fine milled head, and is seen in the Frontispiece in front, and in fig. 20 behind the compound body. The manner in which the screw acts varies in different instruments. In some it has a conical point, which presses against a slit in an inner tube; in others it acts by means of a lever.

OPTICAL PARTS.

These consist, as has been stated, of the mirror, the object-glasses, and the eye-pieces.

The Mirror.—The mirror, as will be seen by reference to the Frontispiece and to fig. 20, is circular. It has two silvered glasses, one concave and the other plane. The effect of the concave side is to cause the rays of light to converge, whilst the plane mirror reflects them parallel to one another. The mirror is capable of being moved by joints in every direction, and should be made so as to slide up and down the bar, in order that the rays from the concave surface may, if necessary, be brought to a focus upon any object on the stage.

The Object-glasses.—The construction of a modern achromatic object-glass is shewn in section in fig. 22.

Fig. 22.

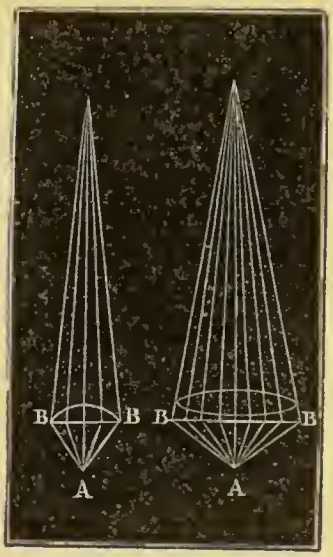
An object-glass, such as is there represented, consists of three plano-convex lenses with the plane side towards the object; each lens is, in fact, compound, consisting of a double convex lens cemented to a plano-concave one, the two component lenses being made of glass of different densities. The manner in which such a glass acts in forming an image is as follows:—The object is placed within the principal focus of the anterior lens, so that a virtual image is formed in front of the lens; the rays from this image fall on the second lens, but the image is so near, even to the second lens, as to cause the formation of a second virtual

image; the rays from this second virtual image fall upon the posterior lens, and are refracted by it so as to form an inverted magnified image behind the whole combination, which last image then is further magnified by the eye-piece. In some achromatic object-glasses of low power, only two compound lenses are employed, and in some of high power four are used. One of the lenses, morever, instead of being doubly-compound is sometimes triple; that is, it is composed of a double concave lens between two double convex lenses.

The object-glasses of the compound microscope are not made perfectly achromatic. Achromatism, perfect, both theoretically and practically, has, indeed, not yet been attained; but, irrespective of this question, object-glasses are purposely constructed so as to project the image formed by the blue rays beyond that formed by the red. This is called over-correcting the object-glass as to colour; the reason for it will be explained when we come to speak of the Huyghenian eye-piece.

Angle of Aperture.—The angle of aperture of an object-glass is the angle formed by the extreme rays of the largest pencil of light which the object-glass is capable of transmitting.

Fig. 23. Fig. 24.



In figs. 23 and 24 are represented two object-glasses, having different angles of aperture; and it will be seen that the pencil of light received by the object-glass in fig. 23, is much larger than that received by the one in fig. 24. If we suppose the two object-glasses to be of equal magnifying power, the images formed by them will be of equal size; but on account of the much larger quantity of light which is collected from every point of the object by the object-glass of larger aperture, minute pores, striæ, or other markings, will be rendered visible by the latter object-glass, which the other would fail to exhibit. Extent of aperture is, therefore, a point of the utmost importance in the construction of object-glasses, and one in which very

rapid progress has been made of late years. Mr. Ross' achromatic combinations of 1-8th of an inch focal length, are made to transmit angular pencils of 175°.

In connection with the object-glass it is necessary to mention the adjustment which is made for viewing covered objects. When an object-glass has been corrected for spherical aberration, so as to afford a perfect image of an uncovered object, it is found that if the object is covered with a piece of thin glass, the correction of the aberration is so far interfered with as to render the performance of the object-glass defective. This effect of the thin glass is remedied by an arrangement by which the distance of the anterior lens of the combination from the other two is rendered capable of being varied within certain limits sufficient to correct the error in the aberration caused by the thin glass. The adjustment is effected by turning a screw collar at the upper end of the object-glass; and Mr. Ross, by whom the arrangement was invented, gives the following directions with regard to it:—

"When an achromatic object-glass for a microscope has its aberrations corrected for viewing an uncovered object, the correction will be nearly the same, whether the object is seen by the light reflected from its surface as an opaque, or by its intercepting transmitted light as a transparent one, if these objects are properly prepared and illuminated; but if it be necessary to cover the object with glass or talc, or to immerse it in a fluid, the aberration caused by the refractive and dispersive power of the interposed medium deteriorates the performance of the object-glass. The adjustment which is given to object-glasses of high magnifying power, and transmitting large angular pencils of light, is for the purpose of compensating the aberration resulting from the various states in which an object may be placed. To effect this there are two lines on the external part of the object-glass; against the upper line is engraved *uncovered*, and against the lower, *covered;* there is also a small square piece of brass, or tongue, screwed into a morticed hole, with a single line upon it, as shewn in fig. 25. Immediately above the lines is a projecting milled edge, which may be moved independently of the other part of the object-glass, giving

motion to the parts marked *uncovered* and *covered*, so that either of the lines may be made to coincide with that on the tongue. This motion has the effect of separating or bringing nearer together the lenses which compose the object-glass. When the line against which *uncovered* is engraved coincides with that on the tongue, the adjustment is perfect for viewing an opaque or uncovered object; but when the line against which *covered* is marked coincides with that on the tongue, the object-glass is in adjustment for viewing an object covered with glass or talc 100th of an inch thick. If the glass or talc is less than 100th of an inch thick, then the mark on the tongue should be between the marks *covered* and *uncovered;* and if it exceed 100th, then the mark on the tongue should be without the mark against which *covered* is engraved. This adjustment must be tested experimentally, by moving the milled edge so as to separate or close together the combinations, and then bringing the object to distinct vision by the screw adjustment of the microscope. In this process the milled edge of the object-glass will be employed to adjust for character of definition, and the fine screw-movement of the microscope for correct focus."

Fig. 25.

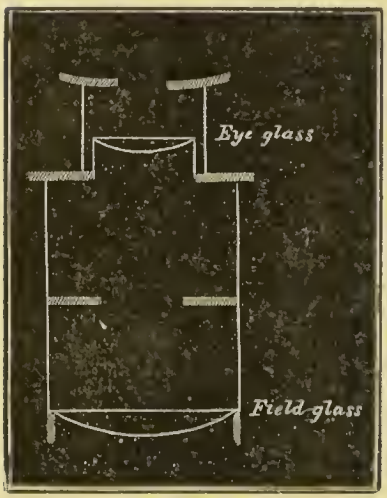
In a late Number of the *Microscopical Journal*, Mr. Wenham has called attention to the great importance of this adjustment, and has given the following practical directions upon the subject :—"When an object is uncovered all that is necessary is to set the glass to the mark *uncovered*, which, in a good object-glass, is placed with great accuracy. When the object is covered, select any dark speck or opaque portion of the object, and bring the outline into perfect focus ; then lay the finger on the milled head of the fine motion, and move it briskly backwards and forwards in both directions from the first position ; observe the expansion of the dark outline of the object, both when within and when without the focus. If the greater expansion or coma is when the object is without the focus, or farthest from the objective, the lenses must be placed farther asunder, or towards the mark *uncovered*. If

the greater coma is when the object is within the focus, or nearest to the objective, the lenses must be brought closer together or towards the mark *covered*. When the object-glass is in proper adjustment, the expansion of the outline is exactly the same both within and without the focus."

The object-glasses furnished with the compound achromatic microscope vary from 2 inches to 1-12th of an inch focal length. The most generally useful ones are the 2 inch, 1 inch, and the $\frac{1}{4}$ inch.

Eye-pieces.—The eye-piece usually employed with the compound achromatic microscope is that which was invented by Huyghens, and is called the Huyghenian eye-piece. A section of it is represented in fig. 26. It consists of two convexo-plane lenses, placed with the plane sides towards the eye at a distance from one another equal to half the sum of the focal lengths of the two lenses, the lens of shortest focus being nearest to the eye. This eye-piece was designed by Huyghens, for the purpose of diminishing the spherical aberration in an astronomical telescope by dividing the deviation of an excentrical pencil between the two lenses, but it was afterwards found that its construction also fulfilled the condition of achromatism of an excentrical pencil.

Fig. 26.

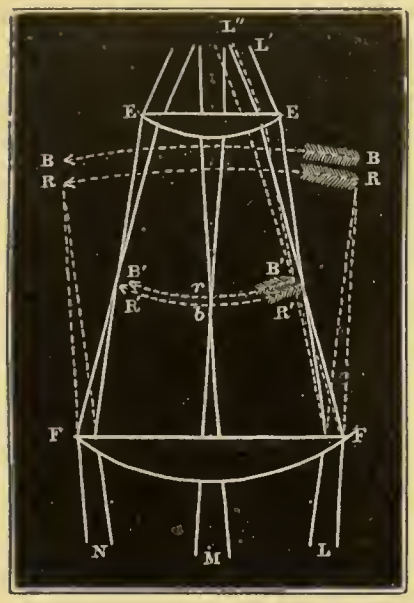


The compensation between the two lenses which renders this eye-piece achromatic, is thus explained. The deviation of the axis of a pencil of light produced by a convex lens is greater as the axis is refracted at a greater distance from the axis of the lens; for the axis of the pencil is refracted in the same degree as it would be by a prism whose surfaces touch the lens at the points where the axis of the pencil is incident and emergent, and therefore the deviation is greater as the refracting angle of such a prism is greater. Now, when a pencil of light refracted by the object-glass falls on the field-glass, it is separated by it

into a series of coloured pencils whose axes follow different courses, the deviation of the axis of the red pencil being least, and of the violet greatest. Since the axes of the pencils do not cut the axis of the lenses between the lenses, the axis of the red pencil falls on the eye-glass at the greatest distance from the axis of the lenses, and consequently is most refracted by it; the axis of the violet pencil falling nearest to the axis of the eye-glass is least refracted by it. Thus the pencils from the same point in the object which are least and most refracted by the field-glass are respectively most and least refracted by the eye-glass, and consequently may be parallel where they enter the eye.*

The effect of the Huyghenian eye-piece in connection with the over-correction of the object-glass will be better understood by reference to fig. 27, where FF represents the field-glass and EE the eye-glass, L, M, and N being the two extreme rays of the three pencils proceeding from the centre and ends of the object. If the field-glass were absent, the object-glass being over-corrected would form a series of coloured images from B B to R R, the effect of the over-correction being to project the blue images beyond the red, and these images would be convex to the eye-glass. The field-glass brings the images to foci at B′ B′ and R′ R′, and reverses their curvature, thereby giving them the form best adapted for vision through the eye-glass. By the over-correction of the object-glass, the blue foci B B, are protruded as much beyond the red foci R R as the sum of the distances between the red and blue foci of the field-lens and eye-lens, so that the separation B R is exactly taken up in passing through those two lenses. The field-glass brings the images

Fig. 27.

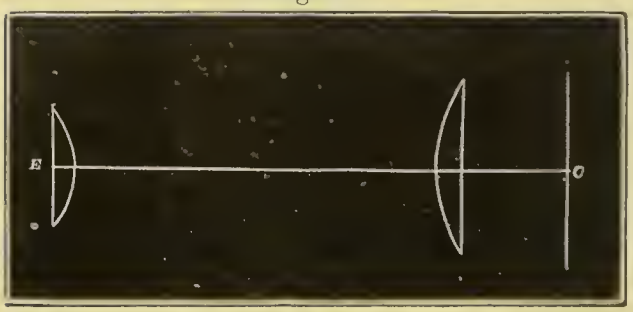


* *See* "Griffin's Treatise on Optics," p. 130.

closer together, and renders the blue images smaller than the red, by the superior refraetive power of that glass upon the blue rays, the effect of whieh is that the blue rays from the image B′ B′ fall nearer to the centre of the eye-glass than the red rays from the image R′ R′, and sinee on account of its spherical figure the refractive power of the eye-lens is less at the margin than at the centre, the result is that the blue rays are less refracted than the red, and the two kinds of rays emerge sensibly parallel. Thus by the over-correction of the object-glass, together with the fact of the spherical error of the eye-lens correeting the chromatic dispersion of the field-lens, an achromatie image is produced to the eye.*

Ramsden's Eye-piece.—This eye-pieee is only used with the compound microscope when it is wished to obtain a measure of the magnified image. It consists of two plano-convex lenses, or, to speak more accurately, of a plano-convex lens and a convexo-plane lens of equal focal length, plaeed at a distance apart equal to about two-thirds of the focal length. The image formed by the object-glass is immediately beyond the field-glass, as at O, fig. 28. This eye-piece is not achromatic, but might be made so by placing the lenses at a distance from one another, equal, or nearly equal to the focal length of either; but if this were done, the image would be coincident, or nearly so, with the surface of the field-glass, and the dust and imperfections on that surface would form spots and flaws in the field of view. We shall have to mention this eye-piece again in speaking of the Micrometer.

Fig. 28.



The Huyghenian eye-piece is sometimes called the negative eye-piece, and Ramsden's the positive eye-piece, for which conflicting and unsatisfactory reasons have been given.

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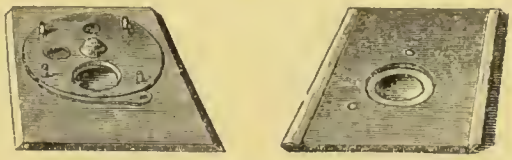
* *See* article *Microscope*, "Penny Cyclopædia."

CHAPTER III.

ON THE ACCESSORY APPARATUS AND CHEMICAL RE-AGENTS NECESSARY FOR THE CONDUCT OF MICROSCOPICAL INVESTIGATIONS IN BOTANY.

The Diaphragm is represented in fig. 29. It consists of a plate of blackened brass having an aperture (in large instruments) of about half an inch. Another circular plate of blackened brass is attached on the underside, which is pierced with holes of different sizes. This second plate revolves, so that each of its holes can be made concentric with the hole in the fixed plate. A spring, having a tooth at the end, is attached to the fixed plate, and the tooth catches into notches on the revolving plate, so as to keep the latter in position when its holes are brought successively over the hole in the fixed plate. The object of the instrument (which is placed under the stage) is to limit the angle of the pencil of light reflected from the mirror.

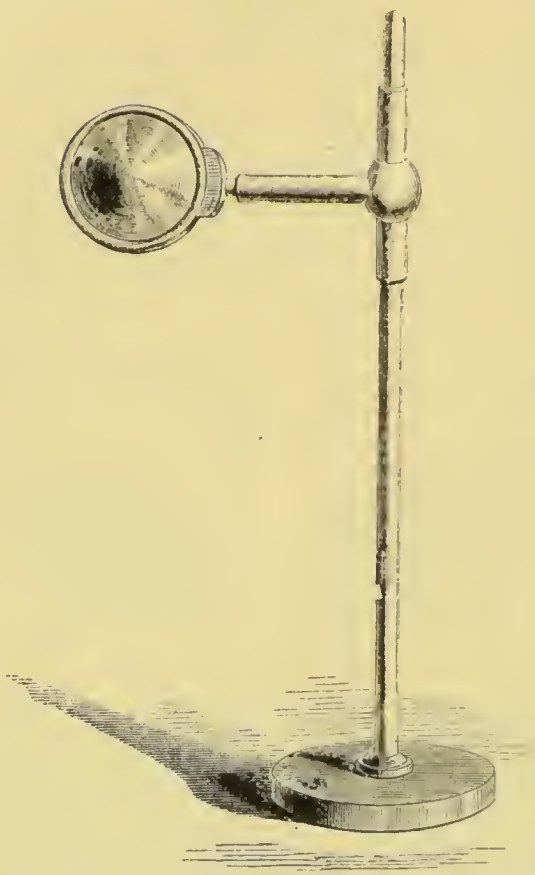
Fig. 29.



Bull's-eye Condenser.—This instrument is represented in fig. 30. It is employed for the purpose of throwing a bright light upon objects on the stage of the microscope, and consists of a plano-convex lens fixed to a stand. The lens is placed before a lamp or candle, so as to cause the rays from the source of light to be brought more or less nearly to a focus upon the object ; the convex side must be turned towards the lamp, as otherwise the spherical aberration is so great as to interfere materially with the condensation of the light. The lens is moveable up and down by means of the piece of brass tubing which slides on the upright rod, and it is attached to an arm of brass,

which slides in the horizontal piece of brass tubing seen in the figure. The lens is also moveable on a joint at the end of the arm, by which, in connection with the other movements before mentioned, it may be brought to bear in any position upon an object upon the stage.

Fig. 30.



Achromatic Condenser.—This is an instrument employed for the purpose of throwing an intense achromatic light upon transparent objects by condensing the rays reflected from the mirror. It consists of an achromatic combination of lenses constructed similarly to an achromatic object-glass, and provided with an arrangement for adjusting the focus. It is placed, of course, under the stage in such a position as to admit the passage through it of the rays reflected from the mirror, so that the light may fall upon the object, and be brought to a focus by the arrangements for that purpose. A simple form of achromatic condenser, devised, I believe, by Professor Quekett, is represented in fig. 31. Delicate markings, not visible under ordinary illumination, frequently exist upon microscopic objects, and in

Fig. 31.

order to exhibit such markings, recourse must be had to the achromatic condenser.

Polarizing Apparatus.—Before describing the apparatus employed for polarizing light, it is necessary to explain what is meant by the term *polarization.*—When a ray of light has been subjected to reflection or refraction under certain peculiar conditions, it acquires peculiar properties, and is said to be *polarized.* The term is derived from a supposed analogy to the poles of a magnet, and although not happily chosen, is now too well established to be altered. Light may be polarized by reflection, by simple refraction, by double refraction, and by absorption.

Double refraction is a property possessed by certain crystals and other transparent bodies, of separating a ray of light passing through them into two portions. By absorption is meant the property possessed by some transparent media of stopping or absorbing part of a ray of light, and transmitting the remainder.

We have stated that, according to the modern theory, light is caused by undulations produced by the vibrations of the particles of an ethereal medium. According to this theory, common or unpolarized light is produced by the vibration of these particles in more planes than one, two of these planes being at right angles to one another, the particles vibrating first in one plane, and then changing their vibrations to another. Polarized light, on the other hand, is produced by the vibration of the particles continually in one plane.

The different properties of common and polarized light, will be seen by reference to the following table, taken from Dr. Pereira's "Lectures on Polarized Light."

A ray of common light.	*A ray of polarized light.*
1. Is capable of reflexion at oblique angles of incidence in every position of the reflector.	1. Is capable of reflexion at oblique angles of incidence, in certain positions only of the reflector.
2. Penetrates a plate of tour-	2. Penetrates a plate of tour-

A ray of common light.	*A ray of polarized light.*
maline (cut parallel to the axis of the crystal) in every position of the plate.	maline (cut parallel to the axis of the crystal) in certain positions of the plate, but in others is wholly intercepted.
3. Penetrates a bundle of parallel glass plates in every position of the bundle.	3. Penetrates a bundle of parallel glass plates in certain positions of the bundle, but not in others.
4. Suffers double refraction by Iceland-spar, in every direction except that of the axis of the crystal.	4. Does not suffer double refraction by Iceland-spar in every direction except that of the axis of the crystal. In certain positions it suffers single refraction only.

Polarized light cannot be distinguished by the naked eye from common light. In order to ascertain whether light which has been submitted to reflexion or refraction has undergone polarization, it is necessary to make use of an instrument called a polariscope ; this consists of two parts ; one for polarizing the light, which is called the polarizer, and the other for examining the light, which is called the analyzer, or test. There is no essential difference between the two, although in adapting them to the microscope, the parts, of course, have to be differently mounted. The instrument usually employed with the compound microscope consists of a prism of Iceland-spar, represented in fig 32, which is placed under the stage as a polarizer, and a similar prism placed over the eye-piece as a test. The prism is an oblique rhombic prism, and is first divided into two wedges in the direction shewn by the dotted line, and the two wedges are afterwards joined together with Canada-balsam. Iceland-spar is a doubly refracting substance ; but the effect of the Canada-balsam is to separate the two images to such an extent that one only is seen through the prism. Two thin plates of tourmaline may also be used as the polariser and analyzer respec-

Fig. 32.

tively, in which case the light is polarized by absorption, one of the polarized rays which constitute common light being absorbed by the tourmaline, and the other transmitted; or, in other words, the vibrations of the ether in one plane are stopped, whilst the vibrations in the plane at right angles are unaffected. The objection to tourmaline plates is, that unless they are very perfect, the polarized ray becomes coloured in passing through them. Dr. Herapath has lately recommended crystals of sulphate of iodo-quinine to be used instead of the Nichol's prisms, or plates of tourmaline, and these crystals are now sold by opticians at a price considerably less than that of the ordinary polarizing apparatus. Ample directions for manufacturing the crystals are to be found in the "Microscopical Journal," vol. i., p. 83.

When light reflected by the mirror through the polarizer is examined by the analyzer, it is found that upon revolving either the one or the other, the light is twice completely stopped in each revolution, and the field of view consequently darkened. This results from the property possessed by polarized light of passing through the analyzing crystal when the axis of the polarizer and the analyzer coincide, and of being stopped when these axes are at right angles to one another.

In order to exhibit the coloured phenomena of polarized light, it is necessary to interpose between the polarizer and analyzer a thin plate of some doubly-refracting substance, and which is called the depolarizer: the depolarizer divides the polarized ray into two, that is, it produces two systems of waves polarized in planes at right angles to each each. One of these systems of waves traverses the depolarizer more slowly than the other, and thus the two are made to intersect one another, or, as it is said, to *interfere*. Now, when two systems of waves interfere, colour is generally produced, but this is not the case when the vibrations of the interfering waves are in planes at right angles to each other; since, therefore, the effect of the depolarizer is to produce two *rectangularly polarized* rays, it is necessary to make their planes of polarization coincide, and this is done by the analyzer.

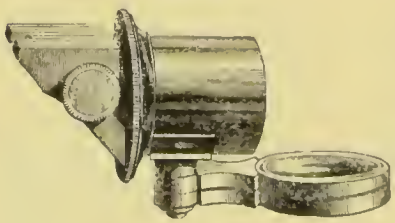
Polarized light is of great use to the microscopist, by enabling

him to discover the existence, in many tissues, of differences of density which would be inappreciable under ordinary illumination. Unequal density being accompanied by the property of double refraction, these tissues when examined with polarized light exhibit the phenomena of coloured polarization. It sometimes happens that the doubly-refracting property of the tissue is not sufficiently powerful to produce colour, and in such cases it is necessary to place a thin plate of selenite under the object. For convenience in use, the plate is sometimes cemented to a piece of plate-glass, and covered by a piece of thin glass joined to it by Canada-balsam, and the apparatus is called a Selenite-stage.

The polarizing apparatus produces beautiful appearances in all irregularly laminated cells. All cells in which the thickening substance is laminated exhibit the cross which is seen in grains of starch; the pits of the wood of Coniferæ and the ducts of the albumen of Phytelephas, or Phœnix dactylifera, exhibit, when seen from above, the same cross. All bast-cells, when viewed separately, or in longitudinal section, shew beauful colours. Wood-cells, such as those in the vascular bundle of Caryota urens, are also very beautiful.

Delineating Apparatus. — The instruments employed for making drawings of microscopical objects are the Camera lucida, the neutral tint glass, and the steel disc, or Sœmmering's mirror.

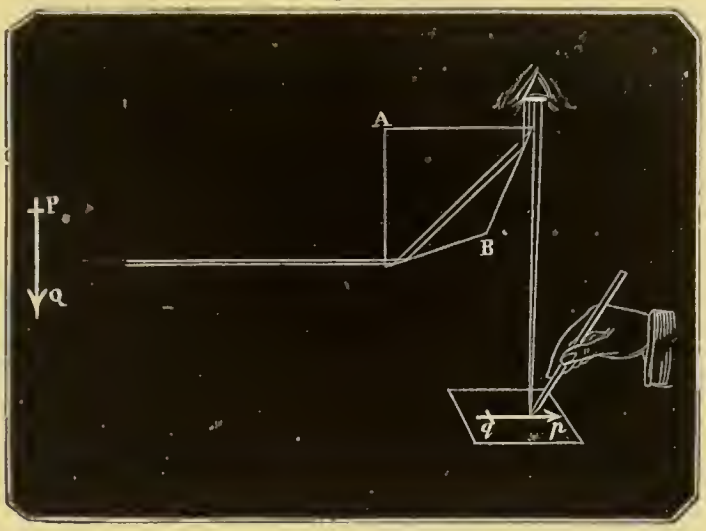
Fig. 33.



Camera Lucida.—This instrument is represented in fig. 33. It consists of a quadrilateral prism of glass mounted in a brass frame, and attached to a short tube, by which it is fitted over the top of the eye-piece. A section of the prism is represented in fig. 34. The rays proceeding from an object, P Q, after passing through the object-glass and eye-piece, fall nearly perpendicularly upon the first surface, are totally reflected at the contiguous surface, and again totally reflected at the next surface, emerging finally nearly perpendicularly to the fourth surface. The eye is placed at the edge

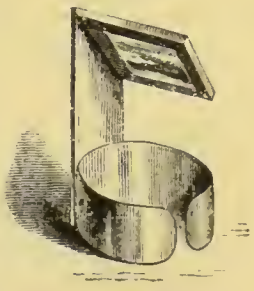
of the prism, in such a position that the pencil emerging from the prism occupies one part of the pupil, and the rays from

Fig. 34.



the drawing-pencil the other part; by this means an image of the object is seen on a sheet of paper at the same time with the point of the pencil, and after a little practice the object may be traced upon the paper. By the use of the two reflexions, the inversions correct each other, and there is the same relative direction of parts to the eye in the image on the paper as in the object. A lead pencil is generally used with the Camera lucida; but if an object is very delicate, it will be found more convenient to use a very fine camel's-hair brush, such as are sold by Messrs. Winsor and Newton, in Rathbone-place, for microscopical drawing. The colour may be either Sepia, Indian ink, or Prout's brown: the latter is the best.

Fig. 35.



Neutral Tint Glass.—This consists of a piece of neutral tint glass mounted in a frame in the manner shewn in fig. 35, and having a ring of brass, by which it is fitted over the eye-piece. It is used like the Camera lucida. When an outline only is required it answers very well, and there is no difficulty in seeing the pencil, but the image is far less bright, and not so well defined as by the

Camera lucida, and it is necessary to shield the paper from the light to a much greater extent than is necessary with the latter instrument.

The Steel Mirror.—This is represented in fig. 36. It is fitted to the microscope by a brass ring, in the same manner as the neutral tint glass. The mirror is smaller than the pupil of the eye, so that the rays of light from the paper enter the portion of the pupil which is unoccupied by the mirror, and by this means the image and the pencil may be seen at the same time.

Fig. 36.

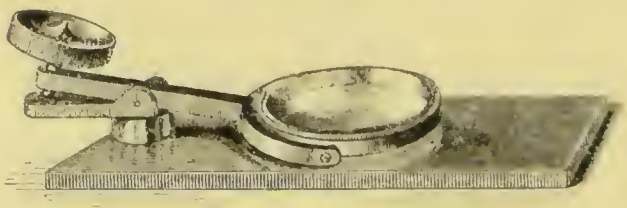
In both the neutral tint glass and the steel mirror, the reflector is placed at an angle of 45°, and there is only one reflexion. The inversions, therefore, are not corrected as in the Camera lucida, but this does not give rise to any practical difficulty in the use of these instruments.

Micrometer.—This is an instrument used for measuring microscopical objects. The most convenient form for the compound microscope is that of an eye-piece having a ruled glass fixed at the point where the image is formed, so that the scale and the image may be seen at the same time. If the negative eye-piece be used, the ruled glass is placed between the lenses, but if the positive eye-piece be employed, the scale is placed in front of the field-lens. Fig. 37 shews a section of a Ramsden's eye-piece, the ruled glass being screwed in beneath the field-lens in the manner represented in the figure.

Fig. 37.

Compressorium.—This instrument, shewn in fig. 38, is used for compressing vegetable tissues when they are not thin enough to be sufficiently transparent. It consists of a plate of brass with a hole in the centre, into which a circular piece of plate-

Fig. 38.



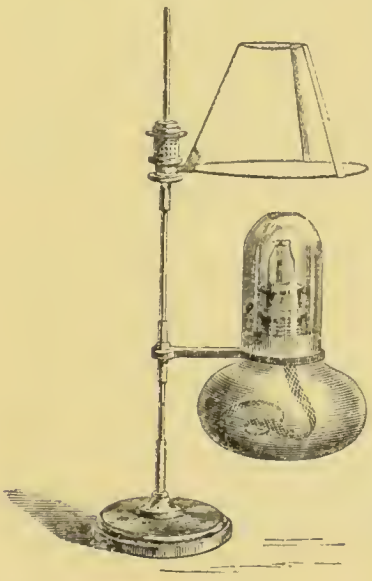
glass is fitted, which serves for an object-holder. This circular piece of glass projects above the metal plate, and a ring of brass carrying a circular piece of thin glass is made to press upon the plate-glass holder by means of the brass arm and screw. The arm may be moved on one side by rotating it upon the circular piece of brass at the left hand of the plate. A gentle pressure with the scalpel on the thin glass covering an object, frequently answers as well or better than a compressorium, which is not a very useful instrument for botanical purposes.

Fig. 39.

Fishing Tubes.—These are made of glass, and are shewn in fig. 39. They are made for fishing up animalcules, but they will be found very useful for taking up Desmidieæ and other minute Algæ.

Lamp.—The lamp called the Cambridge lamp is one of the most convenient for using with the microscope. Sperm oil should be used, the cheaper oils being quite unfitted for burning with this kind of lamp. Care should be taken to ascertain that the glass chimney is sufficiently contracted at the shoulder, so as to create a sufficient draught to ensure a clear flame.

Fig. 40.

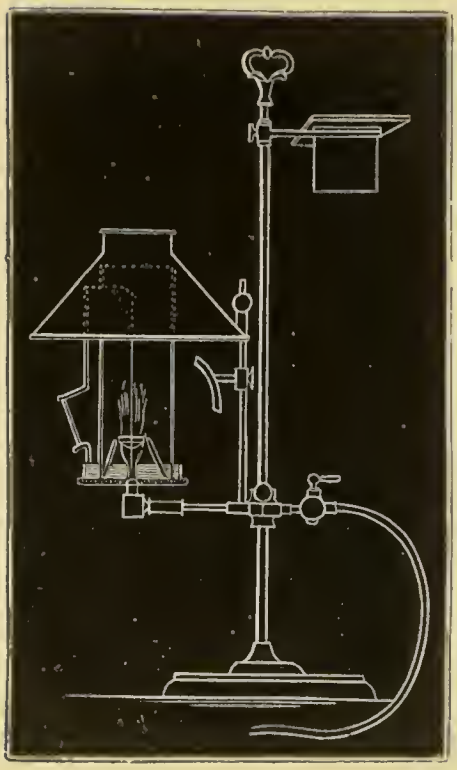


A small and very convenient form of lamp for burning camphine is furnished by Messrs. Smith and Beck, and is represented in fig. 40. This lamp gives an intense and beautifully clear light, but it is, I believe, somewhat more expensive than a Cambridge lamp.

A Palmer's candle, or large wax candle, affords very good

light; but these should be provided with a shade, as otherwise the flickering of the flame from currents of air in the room is very tiresome to the eye. For persons whose houses are supplied with gas, Highley's achromatic gas-lamp, represented in fig. 41, will be found a most convenient and useful apparatus. It consists of a stage supported by a tube and socket, and carrying an Argand burner. A metal cone rises to the level of the burner, and is about an eighth of an inch from its outer margin, by which means a bright, cylindrical flame is procured. A Leblond's blue glass chimney is placed over the burner, which corrects the colour of the flame, and this is further rectified by a disc of bluish black neutral tint glass placed obliquely in front of the chimney. Parallel to this disc, and behind the chimney, is placed a metallic reflector, which concentrates the light.

Fig. 41.



Dissecting Instruments.—The instruments necessary for botanical dissections, are razors, scalpels, scissars, and needles. It is advisable to have two razors, one with the blade hollow on both sides, which is the usual form of razors, and another with one side ground perfectly flat. The flat razor is used when the object is placed in a slit cork and a section made by means of the machine which will be hereafter described. The hollow-sided razor is used when the section is made with the unassisted hand.

Scalpels.—These are made of various forms. The most useful are represented in the annexed figure (fig. 42). It is necessary to have at least two—one of a large size, such as No. 1 or No. 2, and another smaller such as No. 3. No. 4, is a lancet-

shaped knife, sharpened on both sides, and is very useful for making fine sections of minute objects and soft substances.

Fig. 42.—No. 1.

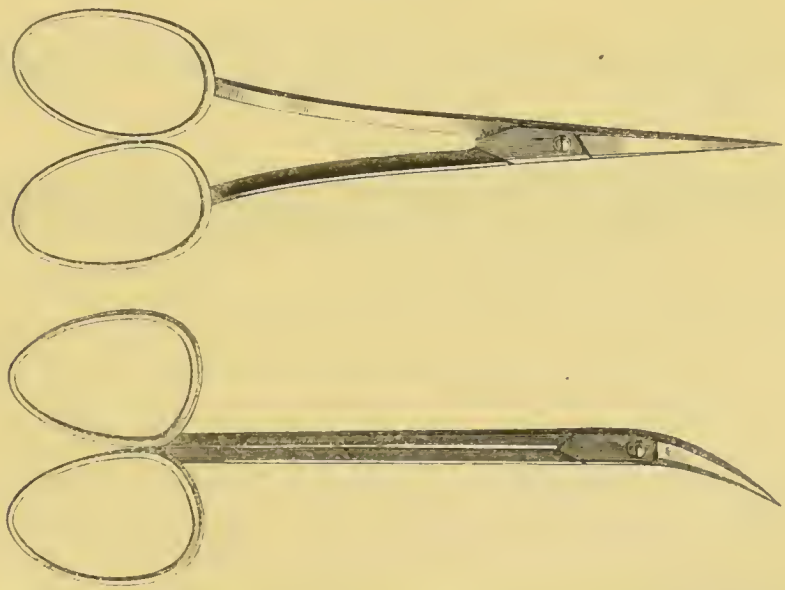
No. 2.

No. 3.

No. 4.

Scissars.—Two pair of scissars should be procured, one straight and another curved, as in fig. 43.

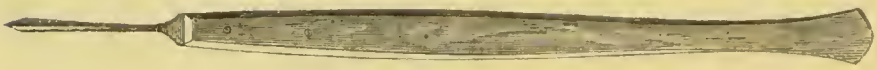
Fig. 43.



Needles.—Different kinds of needles will be found necessary, according to the nature of the plant under dissection. Common

sewing needles, of different sizes, which may be inserted in the handles used for crochet work, are very convenient. Curved needles, such as the one shewn in fig. 44, No. 2, are often very useful, and every observer should be provided with a pair of such needles. When the object to be dissected is very small and delicate, the needles used for operations on the eye, fig. 44, No. 3, are extremely convenient. The knife-pointed needle, fig. 44, No. 1, is well suited for the dissection of objects of a coarser nature.

Fig. 44.—No. 1.



No. 2.

No. 3.

Valentine's Knife.—This instrument is shewn in fig. 45. It was invented by Professor Valentine, with the object of

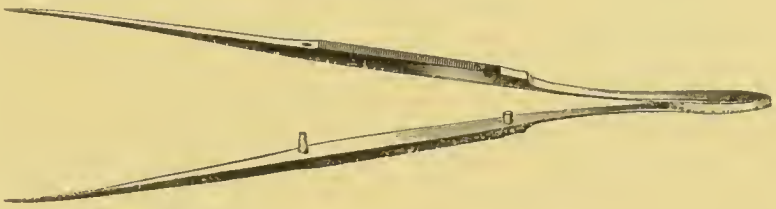
Fig. 45.

facilitating the making of sections of soft substances, such as the liver or the spleen. It consists of two blades, which admit of being brought close together by means of a rivet, which slides in the oblong slit seen beneath the blades. This knife has not hitherto been much used in botanical investigations, but it will be found extremely useful for making sections of soft cellular tissues, such as that of fungi; and very good sections of leaves may also be made with it. The blades should be closed under water, and the section removed from the knife with a camel's-hair brush. In making sections of leaves, the leaf should be

laid upon a flat piece of cork. Mr. Matthews, of Portugal Street, has improved upon the invention of Professor Valentine, by making the two blades entirely separable, which much facilitates the cleaning and sharpening of them. In the knife shewn in fig. 45, one blade may be turned away from the other, but cannot be entirely removed.

Forceps.—One of the best forms of forceps is represented in fig. 46, No. 1. The small rivet on the one side, fits into a corresponding hole on the other side, by which means the points of the instrument are kept accurately together. The crossed forceps, fig. 46, No. 2, are used for holding small objects during

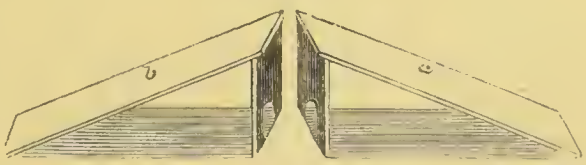
Fig. 46.—No. 1.



No. 2.

dissection with the simple microscope. They are also very convenient for holding the thin glass covers of cells whilst the edges are being anointed with cement. The glass cover turns easily round between the points of the forceps, by merely passing the finger along the outer edge, and thus the necessity of touching the face of the cover, and the consequent dimming of the glass by the heat of the finger, is avoided.

Fig. 47.



Arm Rests.—These are made of deal, and are shewn in fig. 47. They are used for supporting the arms when dissecting

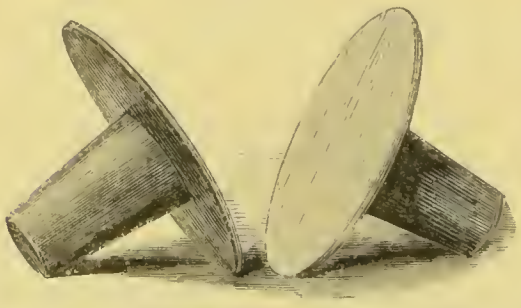
with the simple microscope. The height of the rests must be regulated according to the height of the stage of the dissecting microscope.

Plate-Glass Stage.—This is represented in fig. 48. It is nothing more than a piece of plate-glass, with a rim or ledge cemented at the bottom; it is used when objects are examined in fluid, for the purpose of preventing the fluid from running over the stage when the microscope is inclined. If sulphuric acid or any biting or corrosive re-agent is used, the ledge should be fastened to the plate-glass with gold rivets, as the cement is dissolved by the acid, and the ledge becomes detached.

Fig. 48.

Disks.—My friend Mr. Spencer, of Blackheath, has recommended the apparatus shewn in fig. 49, for cleaning thin glass. It is very difficult to handle glass which is less than about the 1-150th of an inch thick, without breaking it. The disks shewn in fig. 49, may be made of wood or metal. Their surface is covered with chamois leather. If a piece of even the thinnest glass be placed on one of the disks, it may be rubbed clean with the other, and any amount of pressure may be exerted without risk of breaking it.

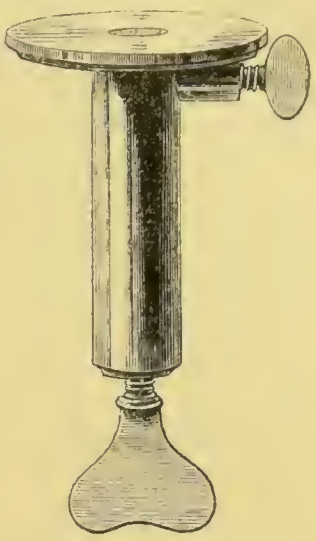
Fig. 49.



Section Instrument.—This is represented in fig. 50. It consists of a piece of cylindrical brass tubing open at the top, and closed at the bottom; a brass disk is fitted inside the cylinder, and is capable of being moved up and down the whole length of the tube by the screw at the bottom. Into this tube is pushed a very soft, perfect cork, which has been divided longitudinally almost to the bottom with a sharp knife. The object from which it is wished to take a thin section is

placed with great care in the slit made in the cork, and the cork is then pushed into the tube, so as to leave the upper surface of the cork just projecting above the edge of the tube; the upper surface of the cork is moistened with a little water, and then with a sharp razor, to which the plate at the top serves as a guiding surface, the thinnest possible lamellæ of cork are cut from the upper surface; with these lamellæ of cork is obtained a thin lamella of the object placed in the slit of the cork, which lamella is removed from the razor and separated from the cork with a fine camel's-hair brush. This mode of proceeding is strongly to be recommended: it is well adapted for all thin objects, and for all small objects which are not too soft; for example, for transverse and longitudinal sections of leaves, of the stems of mosses, of small seeds, and such like things. If the object is somewhat thicker, the cork must be carefully hollowed out a little at the place which is to receive the object. This plan is useless for very soft objects, which can be only cut with the hand. The side screw in fig. 50 is for holding the cork tight in case it does not quite fit the tube. This instrument may be used for making sections of wood.

Fig. 50.



Some large and small camel's-hair brushes are necessary for the purpose of removing the sections of objects on to the slides. For very small objects, none but the finest water-colour brushes are of any use.

In addition to the apparatus which has been already mentioned, the observer should be provided with some glass utensils, such as small bell-glasses, or inverted wine-glasses without legs, for preserving objects from dust. Watch-glasses, of tolerably large diameter, for treating preparations with water, alcohol, or ether, and for boiling thin sections with chlorate of potass and nitric acid, are also necessary, as well as some long, tolerably wide tubes, for warming preparations with water or alcohol, and

for boiling objects with chlorate of potass and nitric acid. Glass slides for mounting objects must also be procured. Earthenware saucers may be used for boiling, and are less liable to crack with the heat than watch-glasses.

A couple of tumblers, or common white saucers, filled with clean water, should always be upon the observing-table; one of these serves for immediate use during the observation, the other for the reception of slides and covering-glasses which have been already used. Slides and covering-glasses are more difficult to clean, and are more easily scratched, when objects become fastened to them in drying.

Some pith of the elder-tree, and some fine linen which has been frequently washed (cambric which has been previously used is the best), are useful for cleaning the object-glasses and eye-glasses. Cloths used for cleaning the glasses of the instrument must never be used for cleaning slides and glass covers; for the latter purpose, a less fine species of linen may be used.

Chemical Re-agents.—The chemical re-agents employed in botanical investigations are principally the following:—

I. Alcohol, which is used for removing air from sections of wood and other preparations, and as a means of dissolving certain colouring-matters, &c.; this is also useful for producing the contraction of the primordial utricle.

II. Ether, which is principally used for dissolving resins, fatty, and other essential oils, &c. This is also useful for removing air.

III. A solution of caustic potash, which is used for the purpose of dissolving fat, is also very useful from its effects upon the contents of cells; it acts also as a solvent upon the cuticle, the intercellular substance, upon wood, and upon cork. This solution often works better after warming.

IV. A solution of iodine (one grain of iodine, three grains of iodide of potassium, one ounce of distilled water) for colouring the cell-membrane, and the contents of the cell.

V. Concentrated sulphuric acid. This is principally used for examining pollen and spores.

VI. Some diluted sulphuric acid (three parts of sulphuric acid and one part water), for colouring the cells of plants which have been previously moistened with the solution of iodine. The object is moistened with the solution of iodine, which is then removed with a fine camel's-hair brush, and by means of a glass rod a drop of sulphuric acid is added, and the object is then immediately covered with a covering-glass. The effect of the sulphuric acid and iodine, as well as that of the iodized solution of chloride of zinc, is not always the same over the whole surface of an object. At the points where the mixture is more concentrated, the colouring is more intense; frequently places remain without any colour. The colour changes after some time; in twenty-four hours the blue is often changed into red.

VII. A solution of chloride of zinc, iodine, and iodide of potassium. A drop of this solution applied to an object placed in a little water, produces the same colour as iodine and sulphuric acid. This solution was first recommended by Professor Schultz, of Rostock; it is more convenient to use than iodine and sulphuric acid, and produces almost the same results; it is, moreover, not so destructive as sulphuric acid; sometimes, however, it fails to produce colour in cases in which iodine and sulphuric acid produce a blue tint in cellulose; iodine and sulphuric acid must, therefore, in many cases, be used in addition to the above solution. The exact prescription for this solution is as follows: Zinc is dissolved in hydrochloric acid; the solution is permitted to evaporate, under contact with metallic zinc, until it attains the thickness of a syrup; and the syrup is then saturated with iodide of potassium. The iodine is then added, and the solution, when it is necessary, is diluted with water.

VIII. A solution of sugar or weak syrup to be used as a re-agent upon nitrogenous matter. The preparation (animal or vegetable, as the case may be) should be saturated with the syrup, and then carefully removed with a camel's-hair brush. A drop of diluted sulphuric acid should then be

applied with a glass rod. If nitrogen be present, the preparation in the course of eight or ten minutes assumes a more or less clear tint of rose-colour. If the colour is very faint it sometimes disappears whilst the object is under the microscope, in which case it is a good plan to place the slide upon white paper, and the colour will then become visible to the naked eye. A weaker solution of sugar may be used for producing contraction of the primordial utricle.

IX. Nitric acid, or, what is better, chlorate of potash and nitric acid. This is used for separating cells. The method of maceration discovered by Professor Schultz, and which is much to be recommended, is as follows: The object (wood, for instance) is reduced in size to the thickness of a lucifer-match; it is then thrown into a long and tolerably-wide boiling tube; to this is added, in a little while, an equal volume of chlorate of potash, and as much nitric acid as is at least sufficient to cover the wood and the potash; the tube is then warmed over a spirit-lamp; a brisk development of gas quickly appears; the boiling-tube is withdrawn from the flame, the oxydizing mixture is permitted to work for about a minute and a half, or three minutes, and the whole is thrown into a saucer with water: the small pieces which adhere slightly to one another are then collected, placed in the boiling-tube, and boiled repeatedly with alcohol, until the latter appears colourless; they are then boiled once more, for the last time, with water. The boiling in alcohol is always advisable, because it not only removes the turpentine, but also carries off the fluid residuum of the acid, which is apt to be injurious to the object-glasses of the microscope. By the help of the simple microscope the cells are now separated from one another with a needle, and selected. The boiling with nitric acid and chlorate of potash should never be carried on in the room where the microscope is kept, because its glasses might be injured by the evaporation which is developed. Thin sections of plants, for instance, of wood or leaves, are warmed for half a minute, or a minute, in a watch-glass; the boiling is unnecessary in

this case; the section is taken out with a little rod, and thrown into a small watch-glass, with water.*

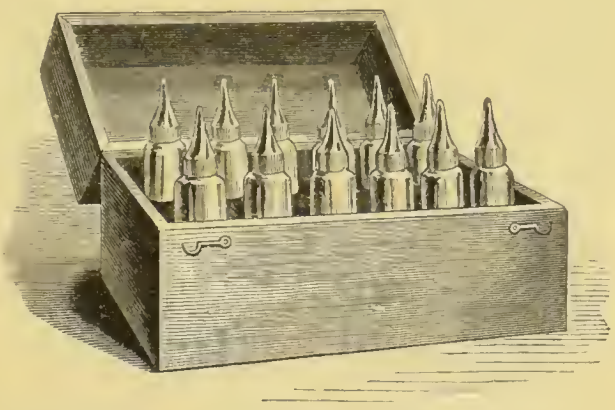
x. Oil of lemons, or any other essential oil for examining pollen and spores.

Fig. 51.

The best mode of applying the chemical re-agents is by means of small bottles, such as the one represented in Fig. 51, which has a capillary orifice, through which the fluid is discharged in drops by the expansion of the air in the interior from the heat of the hand. When it is necessary to refill them, the simplest plan is to place a small quantity of the re-agents in a wine glass, then to warm the bottle over the flame of a spirit-lamp, and invert it into the wine-glass. It is necessary to warm the bottle three or four times, as it is never filled by the first inversion. Care must be taken not to heat the bottle too much, as otherwise it is apt to crack.

Fig. 52 represents a box for holding bottles containing chemical re-agents, such as the one shewn in fig. 51. Boxes of this kind, of different sizes, according to the number of the bottles, and having a gutta percha frame inside, pierced with circular holes, for keeping the bottles erect, are sold, with the bottles, by Mr. Highley.

Fig. 52.



* The boiling with chlorate of potash and nitric acid is an experiment requiring some caution, as the mixture is, to a certain extent, explosive.—Tr.

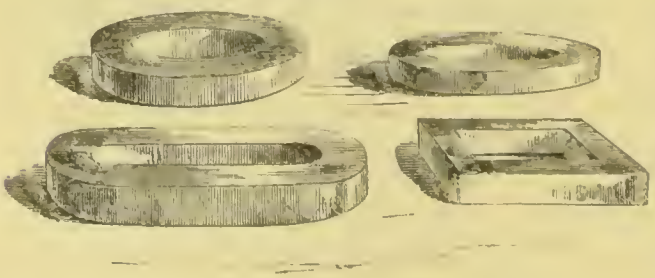
CHAPTER IV.

ON THE PRESERVATION OF BOTANICAL SPECIMENS FOR THE MICROSCOPE.

Opaque Objects.—The majority of botanical specimens which are kept for microscopical examination, are transparent, and require to be immersed in some preservative medium, but there are some objects, such as the seeds of many plants, and a number of the smaller kinds of fungi, which, when preserved entire, it is necessary to examine as opaque objects. These may be mounted on slides, either of plate-glass, mahogany, or ebony. The size of the slides should be three inches in length by one in width. When the plate-glass slides are used, it is necessary to gum a piece of black paper upon them, to form a dark back-ground for the object. The ebony slides are the best for opaque objects, but the wood is expensive, and mahogany, which is a vast deal cheaper, answers the purpose very well, and is, moreover, much lighter, which is an advantage when it is wished to transmit objects by post.

Transparent Objects.—In mounting transparent objects in fluid, it is necessary to be provided with a cell. Cells are manufactured in a variety of ways, which it will not be necessary to describe at length. For botanical purposes, none are more convenient than those made either with glass or cement.

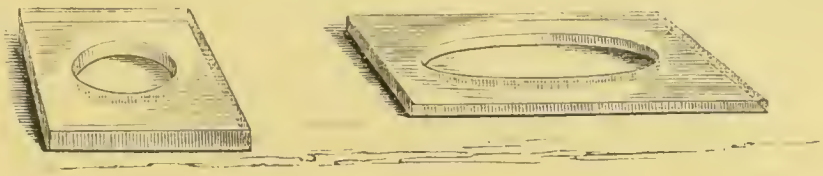
Fig. 53.



Glass Cells.—These are made by drilling holes in pieces of glass, and then

cementing them to plate-glass slides. In figs. 53 and 54 are shewn a number of such cells of different sizes and thick-

Fig 54.



nesses. When it is wished to have a cell for use, with the higher powers of the microscope, it must be made of very thin glass, such as that which is used for covering objects. The thin glass cell may be of the same *shape* as those represented above. It can be made so thin that an eighth of an inch object-glass may be used, provided the cover be thin in proportion.

Cement Cells.—These cells may be made with asphalte, cement, gold-size, liquid-glue, or any material of a similar nature. None is more convenient than gold-size. The easiest way of manufacturing these cells is by the aid of a small instrument called a whirling-table, represented in fig. 55. The instrument is held in the left hand, the glass slide being placed under the two spring clips, and the brass circle which works on a pivot is then made to revolve by means of the milled head underneath the circle. Whilst the slide revolves, a camel's-hair brush dipped in the gold-size or other cement, is held in the right hand in such a manner that the tip of the brush just touches the glass-slide during its revolution. By this means, a thin annular layer of cement is deposited on the slide, which, when dry, serves for a cell. If the cell be not deep enough, a second layer of cement may be added when the first has become dry.

Fig. 55.

Fixing the Cover.—The object is placed within the cell in a drop of the fluid selected for its preservation, and it must then be covered with a piece of thin glass. The edges of the thin glass cover are touched with cement, and in order to avoid air-bubbles

arising in the fluid, the cover should be placed so as to form an angle of about 45° with the plate-glass slide, and then let down very gradually to its horizontal position. The cover may be very lightly pressed, so as to squeeze out the superfluous fluid and to insure the junction of the cement at its edges with the ring of glass or cement which forms the cell.

Preservative Fluids.—The fluids best adapted for preserving botanical specimens are a weak mixture of spirit and water (1oz. of rectified spirit to five ounces of distilled water), a solution of muriate of lime, consisting of one part of dry muriate of lime to three parts of distilled water, or glycerine and water mixed in the proportion of one part of glycerine to two of distilled water. Each of these fluids possesses some desirable qualities, and each has certain defects. The spirit and water has the advantage of being comparatively free from air-bubbles, but it gradually destroys the colour of the specimen, and should therefore not be used when the preservation of colour is an object. It also evaporates rapidly, and consequently it is necessary to take great care that the cover of the cell is hermetically sealed.

The solution of muriate of lime is a very good preservative, but it has not the property of preserving the colour of the object. The great advantage of this fluid beyond its preservative power, consists in its deliquescence. It never dries up, and therefore the cover of the cell does not require such perfect fixing as in the case of spirit and water. This solution must not be used for preserving starch grains, as they swell and become mis-shapen in it. Glycerine and water has the quality of preserving colour to a great extent, and it also evaporates slowly, but there is some difficulty in getting rid of air-bubbles. Mr. Topping recommends, for the preservation of delicate colours, a mixture of one ounce of acetate of alumina and four ounces of distilled water.

Canada Balsam.—This may be used with advantage for preserving sections of wood. The sections must be quite dry, and it is sometimes advantageous to immerse them in alcohol to expel the air. In using Canada balsam, the slide must be warmed, either over a spirit-lamp, or more conveniently by placing it upon a brass table or tripod, fig. 56, with a spirit-

lamp under it. A drop of Canada balsam is placed upon the object, and the thin glass cover, previously warmed, is placed on the balsam, and is either allowed to settle by its own weight, or is gently pressed with the handle of a scalpel. Canada balsam should be kept in a bottle such as is represented in fig. 57.

Fig. 56.

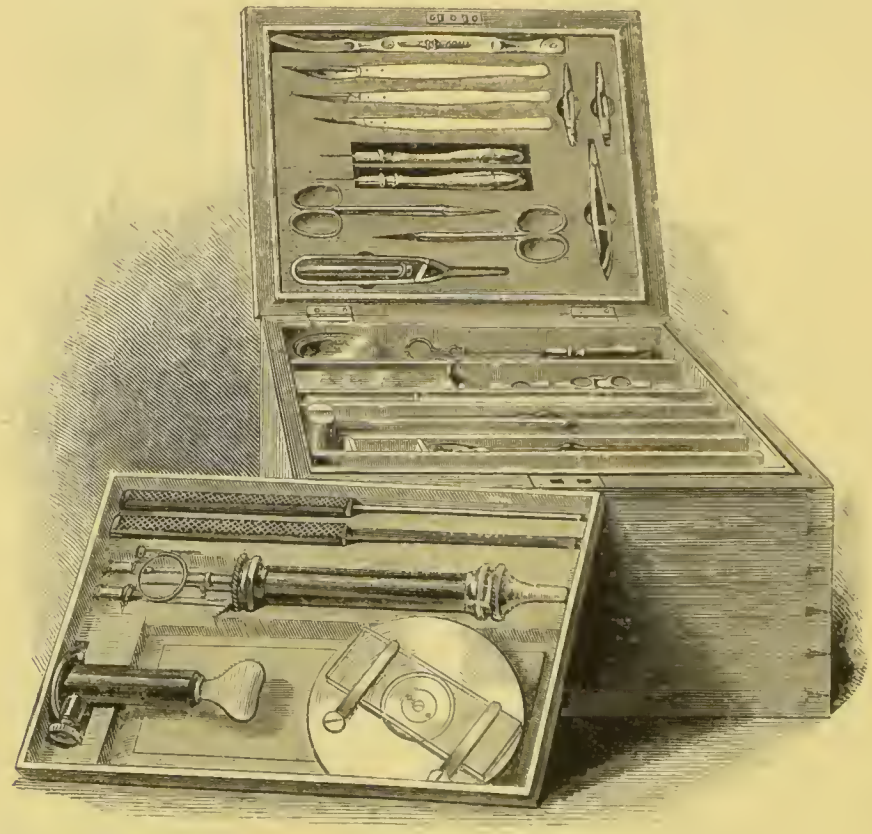
Fig. 57.

Deane's Gelatine.—This is one of the most convenient media for preserving vegetable specimens. It is sold in small bottles, in the shape of a transparent, tough jelly, and the method of use is as follows:—In the first place the bottle must be placed in hot water, by which the jelly becomes perfectly fluid. In order to provide against air-bubbles, it is necessary that the specimen should be quite moist, for which purpose it should be left soaking for some hours in the solution of muriate of lime above-mentioned. Water would do as well for moistening the object; but if the latter should be left long enough for the water to evaporate, it will either curl up or adhere to the glass upon which it is placed, and in either case be spoiled. When the object is thoroughly moistened, the glass slide upon which it is to be mounted should be gently warmed, either upon the tripod or by holding it over the flame of a spirit-lamp; the object is then placed upon the slide, and all superfluous moisture should be removed, either with a camel's-hair brush or a piece of blotting-paper; a drop of the melted gelatine is then placed upon the object with an animalcule tube or common glass rod; the thin glass cover (also previously warmed) is then placed over

the object, and is either allowed to sink by its own weight or gently pressed upon the gelatine.

Castor Oil.—This is a very good preservative medium, and possesses the great advantage of presenting little difficulty with regard to air-bubbles. If the object is dry, there is less trouble with air-bubbles in the use of castor oil than in that of any other medium with which I am acquainted. Objects such as the capillitia of minute fungi, which would require a long pre-

Fig. 58.



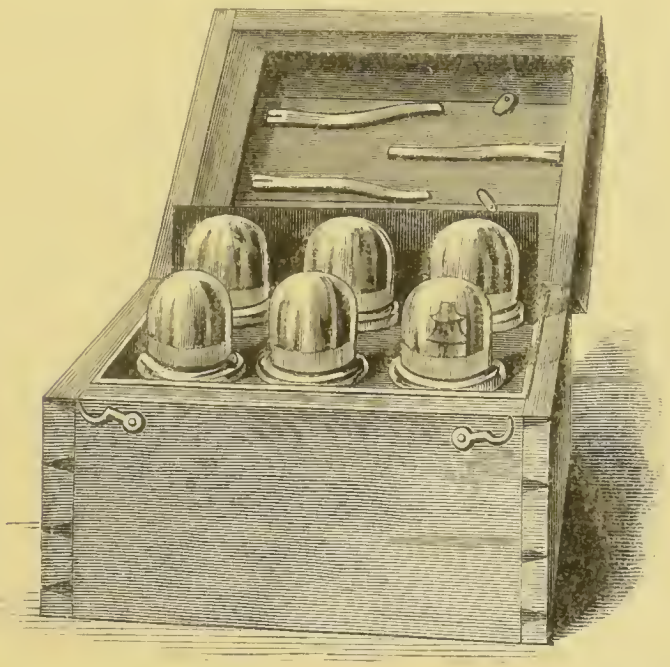
paratory soaking in fluid in order to get rid of air-bubbles, may be mounted at once without difficulty in castor oil. The only objection is, that when the cement cell is used there is some difficulty in properly fixing the glass cover. The oil will sometimes ooze out: when this happens, the best plan is to wait until the oil hardens, which it will do in time, then gently to scrape off the hardened oil, and apply another layer of cement

with a camel's-hair brush and the whirling-table in the usual way.

I may add here that simple distilled water, hermetically sealed, appears to act well as a preservative fluid. I have seen a fragment of a leaf looking fresh and green, which had been kept in a glass cell with distilled water for, I think, upwards of three years.

The names of the objects may be written on the slides with a writing diamond, or on labels attached to the slides. The

Fig. 59.



most convenient labels for slides are the small ones used for ticketing drapers' goods, which are made adhesive at the back, like postage stamps; these labels are made of a variety of fancy patterns, and are sold by parties called *drapers' stationers.*

Fig 58 represents a convenient case, designed by Mr. Highley, for carrying the knives, scissars, whirling-table, and other apparatus used in mounting microscopic objects; and Fig. 59 represents a similar case for holding the bottles containing Canada balsam and the other cements used in mounting, together with a spirit-lamp and mounting plate.

CHAPTER V.

GENERAL RULES FOR THE USE OF THE MICROSCOPE, AND FOR THE ARRANGEMENT OF THE OBJECTS.

One of the principal requisites for microscopical investigation, besides a good instrument, is a proper supply of light. When the position and nature of the apartment can be selected at pleasure, a room should be chosen having windows facing the west or the north; or, what is better, a room with windows towards both those quarters of the heavens. The windows must be as high as possible, since the light received from the horizon is the most favourable; light reflected from a white wall, or the light of white clouds, is very advantageous. The light of scudding clouds fatigues the eye by the rapid change in the intensity of the light, besides rendering necessary a continual change in the position of the mirror. No ordinary observation is possible in direct sun-light; this light is, in the first place, far too dazzling for the eye to bear; and, in the second place, it causes appearances which give rise to the grossest deceptions. In working with the microscope in the forenoon and in the middle of the day, a room lying to the east or to the south must therefore be avoided: by means of white blinds, or curtains, the inconvenience may, to a certain extent, be prevented.

Any person who has regard for his eyes should never undertake microscopical investigations at night; it is true that many objects are seen very beautifully by lamp-light, but this light is far more glaring than day-light. When the light is made to pass through blue glass before reaching the mirror, it bears a greater resemblance to day-light, and is pleasanter to the eye. A piece of white-ground glass, fastened in a wooden frame, and placed before the lamp, will have the same effect. By regulating the light of the lamp in this manner, objects already pre-

pared may be shewn very well by night, but it is hardly possible to make fine preparations with such an illumination; for exact observation, therefore, the day-time only must be selected. In order to intercept the light of the horizon by means of the mirror, the latter is placed at least three feet from the window, the microscope is turned with the mirror towards the light, and the whole instrument, but especially the mirror, is placed in different positions whilst the observer looks through the eye-glass; the light is, in fact, *sought after*: when the field of view appears clearest and brightest, the object which is to be observed is pushed under the microscope.

When it is wished to examine opaque objects with incident light, the microscope may often be advantageously brought nearer to the window. Since for this kind of illumination a much larger quantity of light is necessary, direct sun-light is sometimes desirable; in the absence of this, the condensing lens is used, by means of which the greatest possible quantity of light is concentrated upon the object. In this kind of illumination, the access of light from below, which would interfere with the observation, is prevented by closing the diaphragm. For objects which are altogether opaque, a back-ground which is white, but not glittering, is often advantageous.

The table at which microscopical observations are undertaken must be sufficiently large, and very firm; it must be so arranged that all the apparatus which is ever wanted shall be at hand. Much time is spared by attention to this, and in microscopical investigations time passes only too quickly; moreover, in a very confined space it is impossible to make effectual preparations with the simple microscope. As the chemist requires a special laboratory for accurate inquiries, so the microscopical observer must at least possess a private work-table, which must be used for no other purpose. Roomy cupboards for containing the different apparatus are very desirable additions to this table. Every object intended for investigation should be examined in the first instance with a low magnifying power since by that means a far larger portion of the object is seen, and thus a better impression with regard to the whole is obtained. Should the light be too strong, the plane mirror may

be used instead of the concave one. When the observer has gained as much information as he can with the low-magnifying power, for instance, one of fifty diameters, or, in some cases, even a less magnifying power, the object-glass is changed for a more powerful one. When the most powerful object-glass has been used, and a still stronger magnifying power is found desirable, then a stronger eye-glass is taken. As a general rule, the eye-glass of lowest power should be used, and, if necessary, the magnifying power should be increased by passing from the object-glasses of lower power to those of higher power; but, nevertheless, for seeing with convenience, and especially for drawing, the use of a powerful eye-glass is often not without advantage. As long as the magnifying power can be increased by means of an object-glass, recourse should never be had to the eye-glass, since both the light and the sharpness of outline of the image are necessarily diminished by the use of a powerful eye-glass, which is not the case in using a more powerful object-glass.

In some cases, it is a good plan to shade with the left hand, the eye which looks into the microscope.

When an object is thin enough to be seen with transmitted light, it is first illuminated with light transmitted directly, and it is examined with different, and gradually increasing, magnifying powers; should any details of the image remain undefined, obliquely transmitted light is used, which is insinuated into all the different corners of the object. In some microscopes this is attained by turning the stage round its axis; where this arrangement is wanting, the position of the object must be changed by moving it with the hand. Lines produced by elevations or depressions of the surface, or by want of uniformity in the thickness of the object, or by unequal refraction, always stand out most clearly when oblique light falls upon them at a right angle: where, therefore, a line is suspected to exist, or is only dimly seen, particular attention must be paid to this circumstance. In submitting objects to incident light, the same rule generally holds good, and particular care must be taken, by turning either the stage or the object itself, to concentrate the light in all possible directions upon the object. Object-glasses of very high power cannot be used with incident light, inasmuch as the shortness of

their focal length prevents the light from falling on the object; in this case recourse must be had to less powerful object-glasses, and more powerful eye-glasses. As a general rule, low-magnifying-powers are sufficient when incident light is used.

In most instances, objects are examined under water: it is but seldom, as, for instance, in examing pollen or spores, that it is necessary to observe them in different media, and also when dry. In the case of incident light, water often operates injuriously, especially when the object is not quite covered by it: it is therefore advisable, for certain particular objects, as, for instance, the embryos of grasses, to observe them first without water, and afterwards under water; by placing them under a cover, and adding water with a camel's-hair brush, the object is generally sufficiently and fully immersed. When the object is thick, a slide with a depression hollowed out in the middle of it, may be advantageously employed. When low-magnifying powers are used, it is not necessary that the objects should be placed under a glass cover; in fact, in many cases where it is wished to have the power of turning the object round, or when it is thought that the object may be improved by any additional cutting or preparation, it is very advantageous not to cover it; when object-glasses of very high power are used, the focal distance is so short, that in order to prevent striking the lens against the object, or dipping it in the fluid upon the object-plate, it is necessary to make use of glass covers. When these are used, the fluid in which the object lies frequently becomes lessened by evaporation during the observation, in which case a fresh drop is added at the edge of the glass cover by means of a glass rod, or a clean camel's-hair brush, which may be used when it is wished to add a solution of iodine, or of chloride of zinc and iodine, to objects which are already immersed in water.

When any chemical re-agents are used, whether iodine, caustic potash or any acid, the object should always be covered with a thin plate of glass; in using volatile acids, such as nitric acid and hydrochloric acid, too much care cannot be taken. I avoid using them whenever I possibly can. The vapour of sulphuretted hydrogen has a very injurious effect upon flint-glass, which is used by some opticians for the under side of the object-glass.

The microscope must be carefully protected against gases of this kind, and even against chlorine and such like gaseous matter, on which account, as I have observed before, Schultz's method of boiling the objects with chlorate of potash and nitric acid, must not be undertaken in the room where the microscope is kept.

When the microscope is in daily use, it is a good plan to keep it under a glass shade. When the day's work is over, it is necessary to examine the object-glasses carefully with a magnifying-glass, for it often happens, even to a practised observer, to dip his object-glass in the fluid upon the slide, or to dirty it in some other way; if the glass be only wetted with water, it is of no importance; the consequences, however, may be more serious if the water is permitted to dry upon the lens, especially when the water is impregnated with muriate of lime, inasmuch as after the evaporation of the water, the lime may stick fast to the glass, and give rise to little scratches in the process of cleaning. When the object-glass has become dusty, or dimmed by atmospheric deposits, it may be cleaned with dry elder pith, care being taken to cut off with a clean razor the surface of the pith which has once been used, and thus to obtain a new surface for the further process; the particles of the elder pith are afterwards removed with a clean camel's-hair brush. If the glass has become wet, it is first carefully dried with a clean linen cloth which has been previously washed—cambric or muslin is the best; and afterwards the elder pith is used. If the glass is soiled by any acid, or any other biting fluid, it should be rinsed frequently with distilled water passed through a syringe, and then dried and cleaned in the manner above mentioned. The eye-glasses and the mirror are best cleaned with fine cambric, and also with elder pith. Alcohol and ether should never be used for cleaning the object-glasses, or at least only with great care, since these fluids easily penetrate between the fastening of the lenses, and may reach the cement which unites the crown-glass to the flint-glass. A lens which has been spoilt in this manner can only be rendered fit for use by a practised optician, who can take it to pieces and put it together again.

The greatest cleanliness and accuracy are indispensable for

microscopical investigations; it must be laid down as a rule, always to use the cleanest water, in the cleanest vessels, for moistening the slides. Even with this precaution it is impossible entirely to protect the object from becoming soiled with particles of dust. Extraneous things of this kind will not easily deceive a practised observer; a beginner, however, may be easily misled by them. Water which has been left standing should never be used, since it too frequently contains the inferior sorts of animals and plants; and when different objects are examined one after another, fresh water should be taken for every new object, in order that no particles of the objects which have been previously examined, may be mixed with the water upon the slide. Many errors may be traced to a neglect of small precautions of this sort.

Seeing, as Schleiden very justly observes, is a difficult art; seeing with the microscope is yet more difficult, as our eyes are deprived of all assistance from the surrounding unmagnified objects, and consequently any comparison with them is impossible. This is a fact which we must not only be aware of, but must constantly bear in mind. In microscopic observation two things must be remembered: 1st., That in the microscope, especially with high powers, we see *surfaces* not *bodies*. It frequently happens that in looking upon surfaces, we get a glance into the depths of transparent objects by changing the adjustment, without altering the position of the object; it more often happens, however, that in looking upon such objects, we are unable to make them out to be bodies until we have changed their position, and ascertained their dimensions in three different directions; this, in many cases, from the nature of the object itself, is a matter of great difficulty. 2nd., That we seldom see the objects under the microscope in their natural condition; that we consequently must take into consideration the changes which we ourselves partly produce, either by the medium in which the object is placed, or by the use of the knife, or other influences. Long and thorough practice with the microscope secures the observer from deceptions which arise, not from any fault in the instrument, but from a want of acquaintance with the microscope, and from a forgetfulness of the wide difference

between common vision and vision through a mieroscope. Deceptions also arise from a neglect to distinguish between the natural appearance of the object under observation and that which it assumes under the mieroscope.

In order to be able to recognise extraneous objeets as such, it is advisable to gain an acquaintance with those things which, notwithstanding all precautions, cannot always be avoided. To this class of things belong, 1st., Air-bubbles, which, with transmitted light, generally appear in the form of circles of larger or smaller diameter, with a dark, black-looking rim ; with incident light, on the contrary, their rim appears of a white colour. When the object is under a glass cover, and in contact with it, the larger air-bubbles frequently assume a very irregular shape ; the above-mentioned optical fact is generally, however, by far the best proof of the presence of air, and by it the presence of air may be detected both in and between the cells of plants. 2ndly., Colourless, or coloured fibres of paper, or of linen, woollen or silk textures, left behind upon the object-glasses, from the cloths with which they have been cleaned, and also the hairs which have been detached from the brush. 3rdly., Granular particles of dust, of irregular shape, which are frequently coloured, and are probably produced by the decay of organized bodies.

If it is wished to examine plants or parts of plants, which grow either in or upon the earth, or in water, great attention must be paid to the many organized bodies which are likely to be met with : pains must be taken, by careful observation, to become acquainted with the lower forms of animals and plants : it is necessary, for instance, to be able to distinguish the common forms of infusoria, both those which are provided with siliceous coatings, and those that are not ; also with the yeast plant, the different forms of mould, the Oscillatoriæ, and such like things, in order to be able to separate them from the particular object under consideration.

The epithelial cells of the mucous membrane of the mouth are also objects which may deceive the observer. They occur when the brush is drawn through the mouth previously to bringing an objeet upon the objeet-plate. It is advisable never

to pass the brush through the mouth. When in cutting small objects, the latter are held between the thumb and forefinger, or upon the forefinger alone, it often happens that small fragments of the skin of the finger are cut off at the same time. The observer must learn to distinguish these fragments, as well as the small pieces of cork which he will meet with in sections made between that substance.

The knife sometimes causes deceptions of another kind, when, owing to its not being sufficiently sharp, streaks are left upon the surface which has been cut. In hard woods, such as the wood of palms and of tree ferns, and in very thick albumen, such as that of Phytelephas macrocarpa, this appearance may frequently be observed. The streaks, therefore, must not be taken to be anything belonging to the object, such, for instance, as a layer in the thickening substance. The observer will soon ascertain what the streaks are, if he notice accurately the direction which the knife followed.

Appearances of motion, either usual or accidental, may also give rise to mistakes, and these must, therefore, be learnt. Molecular motion is peculiar to all very small bodies, contained in a thin fluid medium; it consists of a somewhat trembling motion, of these small bodies; it is frequently seen in the interior of pollen grains; it may be observed still better in certain fluids, for instance, milk, when a small quantity is mixed with water, and placed under the microscope, with a magnifying power of from 200 to 400 diameters. When acquaintance is once made with this phenomenon no further deception can be caused by it. The same result follows from accidental currents upon the object-plate, which may take place either by evaporation, or by the mingling of two fluids of unequal specific gravity, or by the dissolving of any salt existing in the fluid. When bodies of small size, and especially round bodies, are examined at the same time as objects of greater thickness, for instance, when the spores and elaters of Liver-worts are examined at the same time as the valves of the capsules, upon the same slide, and under the same glass cover, the former frequently swim about at first in the water, and care must be taken not to be deceived by them. This motion disappears as soon as the fluid comes to

rest. The vibration of the threads of Oscillatoria is, on the other hand, a real motion peculiar to the plant, although it has not yet been explained; the same is the case with the rapid and apparently involuntary movements of the phytozoa of ripe Antheridia, and also with the ciliated spores of Algæ. Moreover, the flowing of the juices of the cell in the cell itself, is particularly interesting, the details of which will be given hereafter.

The "Mouches volantes" belong to that class of deceptions which originate in the eye itself; they are of two kinds: 1st., A slimy secretion from the Meibomian glands; in which case they pass over the field of view of the eye, and are more frequent in the case of persons who are not in the habit of using the microscope.* 2ndly., The shadows of the ramifications of the blood-vessels in a particular part of the eye; since these ramifications are always in the same position, it follows that the form of this phenomenon is always the same. It is seen not only when the Microscope is used, but also (and sometimes more distinctly) when the eye rests upon a snow-flake or a white cloud. This appearance is not confined to one eye. If it should be of such a nature as to cause annoyance, it may be inferred that the eye is in an irritable condition. There is another appearance caused by using too brilliant a light; it manifests itself when the direct light of the sun, or the unsubdued light of a lamp or wax-candle is employed; it consists of spots of different magnitude irregularly scattered over the field of view, which are hardly visible in common day-light; if the eye-glass is turned round, they turn with it, and if the latter is carefully cleaned, they become less; they are caused by dirt upon the glasses, which in very bright light takes the form of spots.

Observations are made less frequently with incident than with transmitted light, but since the latter can only be used for very thin objects, the principal point to be attended to in dealing with opaque objects, is to make such an arrangement of them, as to enable the observer clearly to make out their details. The manner in which the object is divided must be

* It is not generally admitted that *Musca volitantes* are caused by a secretion from the Meibomian glands.—Tr.

regulated and altered according to the nature of the object itself, and the information which it is wished, by the help of the microscope, to obtain respecting it. Firm homogeneous textures, such as wood, must be treated quite differently from delicate objects composed of different organs, such as buds and blossoms; in the case of wood, it is sufficient to take as thin a slice as possible, cut in a certain fixed direction; in the case of buds and blossoms, attention must be paid not only to the direction, but also, particularly, to the point at which the section is made; it is necessary to exhibit an accurate longitudinal section through the middle of the whole bud or blossom, and an equally accurate transverse section made at different heights, in order to ascertain the arrangement of the organs with respect to one another; moreover, the different parts of the organs must be separated and examined by themselves; in cases like this, and especially in inquiries connected with the developement of plants, a dissecting microscope is necessary.

In order to insure success, the kind of knife which is used must be suitable to the object under examination. For wood and hard objects, the best thing to use is a good flat-sided razor, with a broad back. Before cutting, the surface to be operated upon should be moistened each time with a little water; the upper surface in the first instance should be carefully made smooth with another knife, which may be of inferior quality, and the section should then be made by laying the razor quite flat, and drawing it slowly and steadily towards the person of the operator, without removing it from the surface. After every second, or (at the most) every third cut, the knife must be passed over the razor-strop. The thin slices thus obtained must be taken up with a camel's-hair brush which has been previously dipped in clean water, and placed in a drop of water kept ready upon the slide. For delicate or succulent objects, razors with concave-sided blades are much more advantageous; it is unnecessary to moisten the surface of succulent objects prior to cutting them; in other respects they are treated according to the directions given above. The brush with which the objects are brought upon the slide must never be drawn through the mouth, as otherwise the object will be soiled by the

epithelial cells of the mucous membrane of the mouth. Large sections will seldom turn out equally perfect throughout their whole extent. The edges of such sections are generally the most perfect; the size of the sections is of much less importance than the delicacy with which they are made, and the perfect preservation of their cells.

The want of homogeneity in the nature of the texture of some objects, frequently gives rise to difficulties far greater than those which, in other objects, are caused by their minuteness; when, for instance, it is wished to obtain complete and delicate sections, both transverse and longitudinal, through the bark, cambium layer, wood and pith of a dicotyledonous stem, it is impossible to make such sections at one trial, because it will be found that at the points of junction of the different tissues a separation (usually caused by the knife) will take place between the adjoining tissues; it will be necessary in such a case to make many sections, and to choose those which are most perfect. The sharpest possible knife, and slowness and steadiness in making the section, are here especially requisite. As a general rule, it is the best plan to pass from the hard to the delicate portions; a section will sometimes be successful if the knife is placed simultaneously upon the different parts, and in a direction somewhat oblique to that of the wood-cells, or transverse to the course of the medullary rays. In this, as in many other cases, no fixed rule can be laid down, the observer must make experiments for himself, and shape his course according to the nature of the object. The surface from which the section is to be taken must be kept moistened with water.

Succulent or spongy tissues have generally large cells; it is not necessary, therefore, to have thin sections of such tissues, which are always difficult to make. Delicate animal tissues may advantageously be placed in spirit or pyroligneous acid for some days, provided it is not necessary that the tissues should be examined whilst fresh; but I have found that there is little advantage to be derived from treating botanical objects in that manner. It is a good plan, however, in many cases, to saturate delicate portions of animals and vegetables with thick gum-mucilage, and to let them dry slowly in the air.

In dissecting, different methods must be adopted, according to the magnitude of the different objects; objects of large size may be held with the left hand, or with the thumb and forefinger of that hand; very small, or very thin objects, such as the stems of mosses, thin twigs and roots, leaves, small seeds, and such like things, may be placed between cork in the section instrument. Small and very delicate portions of objects, which will not bear the pressure of the cork, may be laid lengthways between the thumb and forefinger, without pressing them, attention being paid to the position in which they are placed. This method is very useful when it is wished to divide a small object into two equal parts; for instance, in holding an ovule, it is often the best plan to place it upon the forefinger, and only to use the thumb for the purpose of preventing it from slipping out of its place. It is often advantageous to moisten the finger a little, as the object then less easily slips about. In cases like this, the section is made very slowly and steadily, the left arm being firmly supported by the table. Sections of small objects thus obtained are examined first without a glass cover, and with a suitable magnifying power. It is often desirable that the object should be turned over, especially when it is wished to improve it by taking a further slice from it; the side from which the fresh slice is to be taken must be carefully examined, as well as the particular spot at which it is to be made. Very small objects may be laid again upon the forefinger of the left hand, in the manner above described, and a fresh cut attempted, which, although not always, is frequently successful. Before cutting, the magnifying-glass should be employed, in order to satisfy the operator that the object is in a proper position for making the intended cut. If, when the section is sufficiently thin, there still remain portions the removal of which is desirable for determining the question at issue, the object should be placed under a dissecting microscope, and the objectionable parts removed, if possible, by the help of a needle or a fine-bladed knife.

For very small seeds, pollen-grains, and spores, I recommend the following process by which beautiful sections may frequently be obtained. A smooth cork is smeared over with thick gum-

mucilage, and the seeds or other small objects are scattered over it and gently pressed into it by the finger. The mucilage is then permitted to dry slowly, and when dry the surface of it is smeared over with similar mucilage, so that the small objects are completely covered. In a day or two the second coating of mucilage will also have become dry. Very delicate sections of the dried mucilage may then be made with a hollow-sided razor, and these sections should be brought under the microscope in a drop of water, by which the mucilage is almost immediately dissolved. Amongst the many objects which will thus be divided, some sufficiently perfect sections are sure to be found. I have examined in this way the very small seeds of Orobanche as well as many different sorts of pollen-grains.

Observations are sometimes disagreeably impeded by the presence of air, which becomes accumulated in the hairy parts of plants, in the intercellular canals, in the vessels, and in wood; it is best removed by placing the object for a few minutes in a small watch-glass filled with alcohol; when taken out of the alcohol it must be put into water, and then transferred to the slide; when it is wished to examine the cell-contents, in which changes are generally produced by the operation of alcohol, the removal of the air may be advantageously effected by the use of the compressorium, which is permitted to operate continuously upon the object, whilst the observer looks into the microscope. In the absence of a compressorium, the handle of a scalpel may be lightly pressed against the glass-cover. I would mention, as an example, the ovules of orchideous plants, which are only fitted for observation when the air has been removed from between the integuments and the nucleus of the ovule.

For transferring objects from one fluid into another a very fine camel's-hair brush should be employed; needles and other sharp instruments should never be used for this purpose, since the object may be easily injured by them. When the object is very small, it will be more easily found if the watch-glass is placed upon a dark back-ground.

For taking up very small objects the fishing tubes mentioned in a previous chapter may be used. The upper end of the tube

is closed by placing the forefinger, previously moistened, over it; the tube is then brought near the object, and upon removing the finger, water will pass into the tube, and the object will be carried into the tube with the water. The object being then brought over the slide upon which it is to be placed, may be gently blown out of the tube on to the slide.

The microscope only affords a view of one surface of an object; when, therefore, *bodies* are subjected to examination, it is not sufficient for a correct understanding of them to examine one side only; a transverse section and a longitudinal section, and, in fact, frequently, many longitudinal sections in different determinate directions, must be carefully examined and compared with one another before the observer can be satisfied that he has made out the construction of the body under observation. That which in objects of large size is attained by the help of the knife, is effected, in the case of very small opaque objects, by examining them on different sides. In examining small bodies which are very transparent, as, for instance, the ovules of Orchideæ, or grains of pollen or starch, the adjustment of the microscope is varied from time to time, by which means the upper side of the object is first brought into focus, then the middle (which may be called an *optical section*, transverse or longitudinal, as the case may be), and, lastly, the under-side. The more perfect the object-glass the more exact is the focal plane, and the more sensitive is the instrument to any small alteration of focus, on which account the observer should always keep his hand upon the fine-adjustment screw whilst he is employed upon observations requiring much accuracy. The sensitiveness above mentioned increases, in good instruments, in proportion to the magnifying power.* By a careful adjustment it is often possible to examine an imperfectly-prepared object, as, for instance, a section which is not sufficiently thin, the alteration of adjustment being rendered necessary by the fact, that in a well-illuminated and perfect microscope the eye cannot perceive anything which lies above or below the focal plane.

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* It increases also with the angle of aperture of the object-glass.—Tr.

The accurate adjustment of an object is judged of by the sharpness of delineation of the image. The adjustment is more accurate in proportion to the delicacy and sharpness of the lines seen upon small objects, and also in proportion to the fineness and clearness of the outline, which should be soft, but well defined. The *Navicula Hippocampus*, and the scales of *Hipparchia Janira*, are very well adapted for enabling a person to judge of the accuracy of an adjustment; the smallest change of focus causes the transverse striæ to disappear; I recommend, therefore, a careful study of these test-objects, in order that the observer may obtain accurate ideas both of correct illumination and of exact adjustment; any person who can accurately arrange his illumination and adjustment for these scales will find no difficulty in any other case.

In examining small round bodies, such as pollen-grains, the position of the objects should be changed, by gently pushing the glass cover so as to cause the bodies to roll about; by this means different sides of the objects are seen, and from the different images presented to the eye their true form is made out.

Small objects should never be compressed between two glass slides, that being too rough a method of proceeding; if, however, it is supposed that anything is to be gained by compression, then it is advisable to use the compressorium. When the compressorium is cautiously used, the observer, by carefully watching what takes place, can gain a knowledge of the changes produced by pressure during the time the compressorium is permitted to work. In certain cases, where, for instance, the question is, whether a particular object is a delicate cell or a drop of some fluid, the compressorium may be of service; since, if a cellular membrane be present, it will burst and discharge its contents as the pressure is increased, whereas the drop, whether it be oil, liquid resin, or any other chemical substance upon the slide, will only change its form.

In examining any object, whether animal or vegetable, it is not sufficient to observe the nature, form, and arrangement of the cells; it is necessary also to pay attention to their contents, which, in the case of plants, are different according to the functions assigned to them by nature. It is necessary, there-

fore, to distinguish—1st. Whether a cell is empty; that is to say, whether it contains air, as is the case, for instance, with perfect vessels and wood-cells; 2ndly. Whether its contents are fluid, with a solid substance contained in the fluid. Another question which arises is, as to the nature of the fluid contents; that is, whether they consist of a homogeneous fluid, or of fluids of different consistencies, apparently not intermingling with one another; the manner in which these fluids are affected by chemical re-agents has also to be considered. Lastly, the solid ingredients of the cell-contents, and their physical and chemical nature, must also be attended to. There are some substances dissolved in the juices of the cell, such as sugar, for example, for which no certain chemical re-agents are known, and yet, perhaps, the red colouring of the contents of ripe pollen-grains, which is often observed to arise upon the application of concentrated sulphuric acid, may be a reaction upon sugar, since Schultz has proved that the effect of sugar and sulphuric acid upon a nitrogenous substance is to produce a red colouring. Gum and dextrine are coagulated by alcohol; the presence of nitrogenous substances is proved, as has been stated, by the use of sugar and sulphuric acid, which produce a rose-red colour, or by a solution of iodine, or of chloride of zinc and iodine, and also by nitric acid, with ammonia subsequently added to it; in these three cases an intense yellow, almost brown, colour is produced. When the presence of oil or resin is suspected, the object should be placed in ether or pure alcohol for some hours, which will dissolve both oil and resin. When the juices of the cell hold any salt in solution, some re-agent must be used which operates upon the salt.

The solid contents of cells consist principally (besides crystals) of starch, inuline, and chlorophyll.* In the case of crystals,

* Inuline is a substance resembling starch, found in some of the Compositæ. It differs from starch in not containing so large a proportion of the elements of water, and in being coloured pale yellow or brown by iodine. The name is derived from the plant Inula. Chlorophyll (from χλωρος, and φυλλον, a leaf), is a substance of a waxy nature, which gives the green colour to plants, and is abundant in the cells of leaves. *See* "Henfrey's Outlines," pp. 12, 13.—Tr.

their form is frequently sufficient to lead to a decision as to their chemical composition; octohedral crystals, so frequent in plants, as well as the long four-sided acicular crystals called *raphides*, are formed of oxalate of lime. Where the form of a crystal does not afford sufficient information, the use of chemical re-agents is often serviceable; carbonate of lime is known as well by the disappearance of its crystals upon the application of sulphuric acid, as also by the escape of carbonic acid in a gaseous form. In this case it is necessary to observe the instantaneous operation of the acid upon the crystal; this may be best observed when the object is laid in a small quantity of fluid under a glass cover, and a drop of acid carefully brought to the edge of the glass cover by the aid of a thin glass rod or capillary bottle; by this means the drop of acid affects simultaneously the whole of the object under examination, and there is time to observe the first working of the acid upon the crystal. I also recommend this mode of proceeding when it is wished to observe the first effect of acid upon a section of a plant saturated with iodine.

Starch is characterized by its assuming a blue colour on the application of iodine; inuline is turned a pale yellow or brown by iodine, and is often not perceptible until iodine has been used; chlorophyll is always green, its grains lose their colour upon being subjected to alcohol; the green colouring-matter which covers them appears to be composed of different chemical ingredients from the grains themselves, which, according to Schleiden, consist of a waxy substance, but which not unfrequently consist of starch. There are many other solid or half solid bodies, which appear in the cells of plants, some with, and some without, any definite form, and which, upon the application of iodine, generally assume a yellow or brown colour, but sometimes shew no change in their colour; the grains in the leaves of some Liver-worts, of *Jungermannia anomala* and *Alicularia scalaris*, are instances of such bodies; the nature of these bodies has not yet been determined. Amongst the substances which are turned brown by iodine, must be reckoned the albumen of the seeds of many plants, for instance, the *Rhinanthaceæ*. A large field is here open for microscopical

inquiry with the help of chemical re-agents. Fatty oil is not unfrequently found in combination with other matter in the cells of plants. The oil is set free by sulphuric acid, and is then seen in drops upon the slide.

The iodized solution of chloride of zinc might be of service in the examination of starch. I have made some experiments with genuine West Indian arrow-root: the grains at first assumed a clear brown-violet colour; the lamination was not very distinct; by gently warming the slide over a spirit-lamp, a blue colour gradually appeared, the laminæ gave way, swelling quite gradually from the outside towards the interior; the inner part of the starch grains sometimes appeared to remain unchanged after the outer laminæ had already become detached; after a quarter of an hour the colour was violet, all the laminæ had become more or less detached, the outer ones were only partly distinguishable, and had become transformed into a granular substance of a blue or violet colour.*

The use of chemical re-agents, however, is not confined to testing the contents of cells, but is also of great importance in obtaining a knowledge of the nature of the cell-wall; for instance, the presence of cellulose in the cell-wall is ascertained by the blue colour which is produced by applying iodine and sulphuric acid, or the iodized solution of chloride of zinc. After maceration in chlorate of potash and nitric acid, all woody and vascular tissue is turned blue throughout its whole extent, by applying the iodized solution of chloride of zinc, which is not generally the case before the application. The oxydizing fluid in this case dissolves not only the intercellular substance but also the woody matter, which, like the corky matter, either altogether prevents the cellulose from being coloured by iodine and sulphuric acid, or causes it to assume a green colour. The real corky matter, on the other hand, is converted into a waxy substance by the oxydizing fluid. In order to remove it, the portions of the plant must be boiled in an earthenware saucer with a solution of caustic potash, and afterwards softened with water,

* On this subject the reader should refer to the papers of Mr. Busk and Dr. Allman in the "Microscopical Journal."—Tr.

which is best done by perpetual boiling. After this process, not only iodine and sulphuric acid, but even a solution of iodine, colours the residuum of cellulose blue or violet. The woody substance is found in the walls of all wood-cells; the corky substance is found in all the cork-formations, and moreover in the so-called cuticular layer of the cells of the epidermis, as, for instance, in the epidermis of Viscum. The cuticle, like the intercellular substance, is dissolved by boiling in alkaline ley; cellulose only becomes a little distended by such boiling. By lengthened maceration after Schultz's method, even the cellulose of the cell-wall is completely dissolved, a fact of which notice must be taken, in order to avoid mistakes as to the construction of the cell-wall. Cavities are frequently met with in macerated cells, although before the maceration the cavity was clothed with a delicate skin; detached twisted threads are also found in places where a continuous membrane formerly existed, which covered the thicker parts, and was arranged in a fibrous manner, as is the case in many bast-cells. In cases like these Schultz's maceration alone must not be relied upon. The real intercellular substance and the cuticle are not turned blue by the iodized solution of chloride of zinc, either before or after maceration.

CHAPTER VI.

ON THE DIFFERENT APPEARANCES, FORMS, AND ARRANGEMENTS OF THE CELLS OF PLANTS, AND ON THE METHODS OF PROCURING THEM AND EXAMINING THEM.

THE cell is the foundation of all the organs of plants; a thorough knowledge of the cell, therefore, in all its different forms, is essentially necessary before any special investigations can be successfully undertaken. A knowledge obtained from books and pictures is not sufficient; it is necessary to become acquainted with the elementary organs of plants from actual observation, and therefore to begin the study of plants with the study of those organs. This chapter contains the most important matters connected with the construction of the cell, together with directions for obtaining objects suitable for observation, and for gaining an intimate acquaintance with them.

To begin with the free Cell.—In the pulp of succulent fruits, for instance, in the ripe currant or raspberry, in the fruit of the snowberry, and also in the leaves of carnations, the cells are so loosely connected together, that it is only necessary to take off small portions of the pulp, or of the parenchyma of the leaves, with a knife, to place it in a little water upon a glass slide, and to spread it out upon the slide as thinly as possible. In this case, a quantity of isolated cells will be found, like small sacs, closed on all sides, containing in the currant and raspberry a coloured, and in the snowberry a colourless, fluid. Every one of these cells generally contains an evident nucleus or cytoblast, that is to say, a granular little body of a round or oval shape, which is sometimes sharply defined and transparent, but more frequently not so; in the interior of this nucleus are often seen one or more very small, round, and generally transparent little bodies, which are called nucleoli. When a detached cell of

this kind is subjected to a solution of iodine, the membrane of the cell becomes of a pale yellow colour, whilst the nucleus and the granular mucilage which frequently surrounds it, and which is generally spread over the whole surface of the cell-wall, assumes a brownish-yellow tinge: if the solution of iodine is now removed with a camel's-hair brush, and a drop of sulphuric acid of the proper strength added, or, what is better, if a drop of the iodized solution of chloride of zinc is employed, the membrane of the cell itself becomes of a beautiful blue colour, whilst the nucleus and the granular mucilage retain their brownish yellow tint. The nucleus is sometimes so transparent, that it only becomes visible upon the application of iodine; it is particularly visible in the tissue of orchids, where it is large and sharply defined. The iodized solution of chloride of zinc does not always produce a blue colour. The effect produced depends upon the strength of the solution and the nature of the cell.

The blue colour assumed by portions of plants, when subjected to iodine and sulphuric acid, has hitherto been always considered as an undoubted chemical reaction upon cellulose; after making many observations during a series of years, I am inclined to think that it is an indication of a certain hydrate of this substance. The effect of iodine and sulphuric acid, for instance, upon very young cells, is, in the first place, to turn them yellow, then reddish, and afterwards violet, and lastly (frequently not until an hour after the application), they become blue; whilst old cells, on the contrary, assume this colour instantaneously. Since sulphuric acid probably operates to withdraw water from the cellulose, it appears to me that the membrane of the younger cells contains a larger quantity of water, or at least that the water is more condensed in them, so that the hydratic condition, of which the blue colour is a characteristic, can only come upon them by degrees. The same blue colouring of the cellulose results upon the application of a solution of chloride of zinc and iodide of potassium, and also upon moistening the section of a plant with an infusion of iodine; in the latter case, however, the object must be permitted to dry, and distilled water must afterwards be added.

By means of the drying, the water appears to be drawn from the cellulose. Withered cells, on the contrary, are no longer rendered blue by iodine and sulphuric acid, nor by the iodized solution of chloride of zinc; the cell-wall of the brown parts of diseased potatoes continues brown, whilst the neighbouring cells, which are sound, assume a beautiful blue colour.

The brown colour produced by iodine in the nucleus and the granular mucilage, and the permanence of this colour when sulphuric acid is added, is usually taken to be an indication of the presence of nitrogen; it is more than probable that both the nucleus and the granular inner coating of mucilage (the primordial utricle) contain nitrogen, although this brown colour alone affords no certain indication of its presence; for both withered cells, such as those in diseased potatoes, and also the cuticle of leaves, are made yellow, or brownish-yellow, by iodine, and by iodine and sulphuric acid, without, as I believe, affording any reason for suspecting the existence of nitrogen. I must, however, observe, that all these last-named parts appear to have a yellow colour before the iodine is used, and that this colour is only heightened by the application of iodine; whilst the nucleus and the granular mucilage are generally colourless in the first instance. Chemistry, unfortunately, here leaves us in the lurch; we can only positively distinguish certain substances in the contents of cells, namely, starch, inuline, chlorophyll, and certain crystallised salts. The rose-red colour which is produced by sugar and sulphuric acid, is always a better indication of the presence of nitrogen; but it is to be observed that the existence of nitrogen, if the quantity be very small, is not always ascertainable by this application, since the colour produced in such cases is too faint to be observable.

When the student has become sufficiently acquainted with the construction of individual cells, and has satisfied himself that they are little closed sacs containing fluid and solid matter; when he has observed that most essential part of the contents of the cells, the nucleus, which is never wanting in young cells, although often concealed by the granular contents of the cell; when he has informed himself concerning the construction of the nucleus, and has ascertained the presence or absence of

nucleoli, and the number of them, if any; when he has observed the relation of the nucleus to the cell, that is, whether it floats freely in the juice of the cell, or is fastened to the wall of the cell, then I recommend him to observe the operation of diluted acid (diluted sulphuric acid or nitric acid) upon the fresh cell; by means of this acid, as well as by alcohol and sugar and water, the inner mucilaginous coating of the cell, which is generally granular, is made to coagulate; it usually becomes contracted like a closed sac, enclosing the solid contents of the cell. Mohl called it the primordial utricle; it is found in all young cells, as well as in the cells of all succulent tissue, in the leaves of the Aloe and Agave, in the fresh leaf of the Liver-wort, in the cells of succulent fruits, in the young parenchyma of the bark of the lime-tree, and other plants; in very thick cells it can seldom be discovered.

We will now consider the further development of the cell, and there are three points which must be particularly kept in view; first, the growth—that is to say, the increase in size of the cell; secondly, the degree and manner of thickening of the cell; thirdly, the arrangement of the cells with respect to one another.

The cell, which at first very frequently assumes the form of a small closed sac, of a more or less round shape, afterwards increases in size in different ways. Sometimes it increases equally on all sides and at all points of its periphery. In this case the cell, if it is only slightly pressed upon by the neighbouring cells, retains its original round form. This form of cell is of comparatively rare occurrence; it is often found in the reproductive cells, in spores and pollen-grains, and also in the pulp of ripe succulent fruits. Cells of the kind now under consideration (that is, cells which increase equally on all sides and at all points of the periphery) are more frequently of a polygonal form, owing to the mutual pressure of the cells; the number of the angles of the polygon depends upon the number and arrangement of the cells which are in contact. Such cells, when cut transversely, frequently appear to be pentagonal, hexagonal, or polygonal. Tissue consisting of such cells has been denominated regular parenchyma. It is very widely distributed; it is found in the

potato, in the pith of most trees, in the roots of orchids, in the leaves of aloes, and in other plants.

The second mode of development of the cell is that in which it increases to a greater extent in one direction than in another; by this means we obtain cells extended either in length or in breadth. Length and breadth must here be considered only with reference to the arrangement of the cells amongst one another; wood-cells may be taken as examples of cells extended lengthways, because they follow the direction of the length of the stem; the medullary rays are instances of cells extended breadth-wise, because they run in an opposite direction. In the stems of succulent plants, as, for example, in the stems of balsams, we find the so-called elongated parenchyma, consisting of rather large, slightly thickened cells. The cambium of dicotyledonous plants also contains narrow elongated cells with very delicate walls. The wood-cells are generally extended, much thickened, and pointed at both ends. In order to examine fully the form of all these cells, maceration may be advantageously employed.

The third mode of growth is that in which the cell increases equally on all sides, but not equally at every point of its periphery. The most elegant form of this kind of cell is the stellate tissue, such as that which is found in the pith of Juncus conglomeratus, and in the leaf-stalk of Musa; it is also found, although less regular in its shape, in many other spongy tissues. In the latter case it is often difficult to make out the particular form of the cells. If their partition walls are very delicate, the iodized solution of chloride of zinc, or iodine and sulphuric acid, by which these cells are generally turned blue, are very useful. Tissue of this nature is traversed by air-passages, which arise from the circumstance of the cells only touching one another at certain spots, frequently of very small extent, and from this circumstance also arises their spongy nature. The epidermis of many plants, such as the leaf of the beech, and the leaves of many ferns, consists of flat cells, the walls of which dove-tail into one another; the prominent part of one cell fitting exactly into a depression in the neighbouring cell; such tissue, therefore, differs from spongy tissue by the absence

of air-passages between the cells. The construction of the periderm of Pinus sylvestris is similar to that of an epidermis of the nature just mentioned.

The increase in thickness of the cell appears to arise from the deposit of solid substances in the interior, upon the original cellulose of the cell-wall. This deposit of new matter frequently appears to take place in the form of a spiral; in very young wood-cells, for instance, in the youngest cells of a fresh twig of Pinus Abies, a most delicate spiral band may be observed in Spring and in Summer: in the older cells it can hardly be perceived. The band which is seen in spiral cellular tissue, the markings in the thickening layers of the liber-cells of Vinca minor, the arrangement of those spots, the thickness of which is less than that of the rest of the cell, and which occur in the thickening substance of the wood-cells of Caryota urens and Hernandia sonora, as well as the disposition of the slit-shaped pores of many wood-cells (those of Cycas, for example), all afford arguments in favour of the thickening substance being deposited in the form of a spiral. There are many cases, however, in which no spiral is visible in the otherwise highly developed thickening layers. This occurs in the albumen of the Date and the Cyclamen, in the so-called Collenchyma of the bark, in the tissue of the leaves of many Liver-worts (such as Jungermannia anomala and crenulata), and generally in those cases where the corners only of the cells are thickened. Spiral and annular thickening layers are generally found in those cases where the cells themselves become considerably elongated after the formation of the layer. Those portions of the cell-wall which are slightly, or not at all thickened, become stretched by this elongation, by which means the coils of the spiral band, or the rings, become further and further separated from one another. When the layers are deposited in a net-like manner this separation takes place only to a very slight extent, if at all; in this case the elongation of the cell takes place before the deposit of the thickening layers. An accurate acquaintance with the mode of developement of the internodes of the stems of Balsams is here very desirable. In the youngest internodes only spiral and annular vessels are found, and in these the coils of the

spiral, or the rings, as the case may be, lie close to one another; as the internode becomes elongated its cells become elongated also. The coils and the rings of the oldest vessels are now drawn far apart from one another, and become still further separated in proportion as the internode becomes longer. New vessels of the same kind are continually being produced during the time the internode increases in length; when the internode has attained its full length, net-like vessels are formed.

The above facts may be observed by following out the developement of the internodes of the young branches of any forest-tree. In the medullary sheath the vessels are principally spiral or annular, which vessels are not usually found in the wood. In the Coniferæ the medullary sheath always exhibits thickened spiral cells instead of pitted wood-cells. A young beech twig just breaking through the scales of a bud, affords a particularly good illustration of the facts above mentioned.

In almost all cells of great thickness, for example, in many wood-cells, there may be seen a manifest lamination in the thickening substance, from which it would appear as if the thickening took place periodically. Observations upon the wood-cells of Caryota urens and Hernandia sonora have convinced me that, contemporaneously with this lamination, the direction of the spiral may also be changed. The existence of the spiral band shews that the thickening substance is not spread equally over the whole wall of the cell; the thickening substance does not consist of spiral filaments; the spiral only points out the spots where the layers are of greater thickness.

Certain spots may be observed here and there, the thickness of which is less than that of the rest of the cell; these thin spots often occur at very regular intervals; they are frequently arranged in the form of a spiral, and are often of a regular shape; at the places where these thin spots occur there appears to be a change in the substance of the cells. Even in cells of slight thickness the thin spots can be seen upon making thin longitudinal or transverse sections, especially by using the iodized solution of chloride of zinc, or iodine and sulphuric acid; for it will then be seen that these spots continue almost colourless, whilst the thicker portions of the cell-wall become

of a beautiful blue colour. I would mention, as examples, the starch-bearing tissue of the potato, the cells of the leaf of Fegatella conica and Preissia commutata, &c. In cells of greater thickness these thin spots appear as pores, or canaliculi. With the exception of the cuticle of certain plants, such as Cycas revoluta, Aloe succotrina, Hakea, &c., these pores only occur at those points where two cells touch one another, in which case the pore of one cell exactly meets the pore of another cell, but each is divided from the other by a thin membrane, an excellent example of which is to be seen in the albumen of the seed of the date. Between the walls of the two cells there is often to be seen a lenticular cavity. The wood-cells of the Coniferæ afford the best instances of this, and the vessels of the tropical twining plants, such as Büttneria and Porana, in which branched canaliculi are often to be found, are well worthy of examination. The tissue in which these canaliculi and lenticular cavities occur is called pitted tissue. In order to become acquainted with the construction of the pits, they must be seen from three sides. In Pinus sylvestris, when a transverse section, or a longitudinal section at right angles to the medullary rays, is taken, the pits are seen sideways; the canalicus of each wood-cell may then be seen, and, between the cells, the lenticular cavity. If a longitudinal section is made in the direction of the medullary rays, the pits are seen from above in the form of two circles, one within the other; the larger circle is the boundary of the lenticular cavity, the smaller circle within it points out the situation of the canaliculus. The latter does not always appear in the form of a circle; in the wood-cells of Cycas it is in the shape of a slit, because here the canaliculus is of that shape. When the canaliculus, as is the case in some specimens of wood, runs into the cell in a conical form, a third circle is seen, which corresponds to the mouth of the canaliculus.

Parenchyma and prosenchyma are distinguished from one another by the extent to which the thickening takes place; the former consists of slightly-thickened cells, not pointed at the ends, and not overlapping each other; prosenchyma consists of cells with pointed ends, whose walls are of considerable thickness, and which overlap one another. Nearly allied to these

are the liber-cells, whose thickening substance, however, is less firm and brittle, but more pliant and tough; a property which renders them of great utility.

Vessels are distinguished from the other cells of plants by the fact, that they are placed in rows one above the other, and are rendered continuous by the obliteration, in whole or in part, of their transverse partition-walls, by which means a tube is formed, which is, to a certain extent, continuous. When the partition-walls of vessels meet one another in a horizontal direction, the wall is generally found to be perforated by a round hole; this is the commonest case. When, however, the walls meet one another in an oblique direction, a scalariform partition-wall is often to be found instead of the round holes; that is to say, a wall with slit shaped cavities lying near one another, and running in the direction of the breadth of the vessel. In the vessels of Alnus, Betula, Corylus, Platanus, Buxus, Thea Bohea, Caryota urens, Ephedra, &c., a double row of round cavities generally appears under similar circumstances in the place of the slit-shaped cavities. Real holes in the substance of the cell-wall are sometimes, but very seldom, met with; as, for instance, in the leaf and stem of Sphagnum.*

The spiral vessel is the true type of vascular tissue; in it the thickening substance is developed in the form of a continuous spiral band: in the annular vessels, on the contrary, the thickening substance is deposited in the form of a ring. In the stems of balsams, the most beautiful transition from the spiral to the annular vessel is to be seen. In the scalariform vessels which occur in the wood of the vine, in the leaf-stalks of ferns, and particularly in the stems of tree-ferns, the coils of the spiral are, as it were, joined together by a border, such border occurring at those spots where the vessel meets with several of the adjoining cells. In delicate, thickened, reticulated cells these borders, which unite the coils of the spiral, frequently occur in greater numbers. In the stems of balsams, all the above-men-

* The apparent perforations in the cells of Sphagnum have been proved to be *real holes*, by observing the passage of animalcules in and out of the cells.—*See* "Quekett's Lectures on Histology," p. 9.—Tr.

tioned forms of vessels, except the scalariform vessels, may be found in the greatest perfection. The most beautiful pitted vessels are to be found in the wood of Laurus Sassafras; the pits and the spiral bands are both present in Tilia Europœa; and the same is the case with the wood-cells of Taxus. The longitudinal section of a scalariform vessel exhibits a pit between each pair of the bars. A good transverse section shews that this pit is slit-shaped, so that the scalariform vessel is most nearly allied to the pitted vessel. Schultz's method of maceration is highly to be recommended for the examination of cells. All succulent stems are particularly well adapted for making observations on the vascular tissue.

The vessels, the wood-cells, and the liber-cells, become filled with air as soon as they are completely formed. The contents of the parenchyma, on the other hand, are fluid, and solid matter appears to be held in solution, or suspension, in the fluid. If a longitudinal section of moderate thickness is made through the bundles of vessels of a fresh portion of a plant, and treated under water, they will appear to be white, if examined with incident light, before the water has time to force its way into the vessels; if they are examined with transmitted light, they will appear black: these appearances are well known to indicate the presence of air. If the section be now placed in alcohol, in order to drive the air out of the vessels, and brought again under the microscope in a drop of water, so that the vessels become filled with water, they will then appear transparent like the cells of parenchyma.

It will have been seen that the cells of plants vary much in their mode of growth, and in the manner in which they increase in thickness. They are joined together by a secretion, which originates in the cells themselves, and passes into the intercellular substance. When thus joined together they form different sorts of tissue, which we now proceed to notice.

Parenchymatal or Nutritive Tissue.—This is characterized by thin-walled cells, the forms and contents of which, however, may be very various; regular parenchyma, consisting of nearly round cells, or of cells whose length is the same as their breadth, is to be found in the pith of most trees; *elongated*

regular parenchyma is found in the pith and in the bark of dicotyledonous plants of rapid growth; stellate parenchyma is found in the pith of rushes; spongy parenchyma in the air-passages of many aquatic plants, &c. The tissue of lichens, as well as of the higher fungi, may also be considered to be a special kind of parenchyma.

The reproductive tissue of plants consists of parenchyma. In the cells of the parenchyma new cells are produced, as well as different sorts of vegetable matters, such as starch, inuline, essential and fatty oils; crystals are also secreted in them. There is, moreover, a kind of primordial parenchyma which serves in the first place for the multiplication of cells, and which possesses the power of producing all sorts of cells. This tissue is found in the earliest rudiments of the embryo, and in the punctum vegetationis of the stem and of the root.

Formative Tissue or Cambium.—This consists of very thin-walled cells, which become transformed gradually, and by certain specified changes, into the different kinds of cells of the vascular bundle. If, whilst the Cambium is occupied in forming new wood on the one side, and new bark on the other, provision is made for its own growth also, it causes a continual increase in the thickness of the vascular bundle on both sides. This is the origin of the annual rings in our forest-trees; since by means of the Cambium, a new layer of wood is formed every year. But, by the same means, the bark also increases yearly. This mode of growth characterises the dicotyledonous vascular bundle. Vessels originate immediately from Cambium cells by the obliteration of the transverse septa in a row of such cells. By the formation of a wood-cell the primordial utricle of the Cambium cell is divided longitudinally into two equal halves; one of these halves becomes a wood-cell, whilst the other continues for some time as a Cambium cell. The perfect wood-cell is pointed at both ends; its wall is generally much thickened and furnished with real pits. Woody parenchyma originates from cell-formation in the interior of a very young wood-cell; the new cells are formed by the transverse division of the primordial utricle of the wood-cell. The original cell is frequently absorbed, but sometimes remains behind, as in the case of the

Vine, where the perfect wood-cell has a spiral band, which is not to be found in the woody parenchyma. This latter parenchyma is found in the Leguminosæ, and in the Oak, and the Beech; in Ulex and Spartium it is furnished with a spiral band. Woody parenchyma may be recognized by the shortness of its cells in comparison with true wood-cells, by the absence of pointed ends, and finally by the contents of the cells which frequently consist of starch and other hydrates of carbon, which are never found in the true wood-cell.

Bast-cells, like wood-cells, take their origin in the longitudinal division of a Cambium cell, but it would seem that more than two cells (generally four) originate from one mother-cell. The bast-cell generally becomes more or less perceptibly elongated; its ends are pointed like those of the wood-cell, and it becomes thickened in a manner similar to this latter cell; it does not, however, always become woody, and it is never truly pitted. Its thickening layers, however, are either penetrated by canaliculi, or have some parts thinner than others, the thinner parts traversing the layers, either in a lattice-like manner, as in Vinca Asclepias, and Urtica, or in a slit-shaped form, as in Caryota and Phœnix. The bast-cells of some plants, such as Abies pectinata, Picea vulgaris, &c., after remaining for many years without becoming woody, and serving as conduits for the sap, are still capable of cell-formation; the secondary bast-cells originate in them, which latter cells generally become woody, and in Abies pectinata, are distinguished by the peculiarity of their form and the number of their ramifications. The Cambium itself never contains starch or other hydrates of carbon; its contents are granular: sugar and sulphuric acid produce a rose-red colour; it is, therefore, rich in nitrogenous substances. The vessels and the wood-cells, when in their perfect state, contain air; the bast-cells, on the other hand, in most plants, are for a long time filled with sap: milky juice is a product of these cells. The developement of Cambium and wood-cells may be best studied in the spring in the roots of Pinus Abies and Larix. The origin of vessels may be traced most beautifully in Dicotyledons of rapid growth; for instance, in the branches of Broussonetia and Paulownia. The Vine is a favourable plant

for studying the development of the bast-cells and woody parenchyma.

Epidermal Tissue.—This tissue is very various, and may be subdivided into the epidermis proper, the epithelium, and the cork. The epidermis proper consists, for the most part, of a stratum of tolerably thick-walled cells. The form of these cells themselves is very various in different plants: in the monocotyledonous plants, such as the grasses, the Irideæ, the Orchideæ, &c., the cells are elongated, and of regular shape; in the leaves of ferns, on the contrary, they are very irregular in shape, being united together almost in a stellate form. In the leaves of dicotyledonous plants they are differently formed in different plants. It not unfrequently happens that the under side of the same leaf has a differently formed epidermis from the upper side. Between these epidermal cells, but more frequently close underneath them, are situated the stomata. With the exception of Marchantia, the stomata are almost always formed of only two cells. In Cycas and some Proteaceæ both these cells lie very deeply buried under a crater-shaped hillock formed of many epidermal cells. In Nerium Oleander they lie in clusters in deep cavities of the leaf formed for the purpose, whilst the smooth upper surface of the leaf has no stomata. The stomata, especially in plants which grow in the air, are generally to be found on the under side of the leaves. In Cycas and Nerium, in the Beech, the Oak, the Alder, &c., they are altogether wanting on the upper side. In the floating leaves of aquatic plants, such as Hydrocharis and Nymphæa, they are to be found only on the upper side. The epidermis is frequently clothed with hairs; these hairs are generally prolonged cells of the epidermis itself. The hairs may consist of one or more cells; in the latter case they frequently end with a cellular knob like the glandular hairs of Pinguicula vulgaris and Polycarena capensis; the stinging hairs of the Urticeæ, on the other hand, consist of only one cell, the very small end of which bears a little knob which is somewhat bent and very easily broken. The scales of Elæagneæ, of certain Bromeliaceæ, &c., belong also to this class; they are, as it were, compound hairs. Branched hairs, not compound, but rather con-

sisting of a single cell, are comparatively of rare occurrence; they are found in certain species of Alyssum, and are even more beautiful in certain Amaranthaceæ, for instance, in the leaves of Alternanthera axillaris.

The epidermis proper, and the parts belonging to it, such as the hairs and the outer side of the cells of the stomata, are clothed with a continuous covering, the product of a secretion of these cells, which is called the cuticle, and which, in my opinion, covers the whole epidermis, although it is not of equal thickness at every point of it. In the young epidermal cells this cuticle is very slightly developed: it is often, in fact, nearly fluid, and afterwards appears as a firm membrane capable of resisting the strongest sulphuric acid. It is particularly beautiful in the leaves of certain Orchideæ (Himantoglossum, Orchis fusca), in the hairs of Monotropa, and of certain species of Borago, &c., where it forms streaks or wart-like elevations. In leathery or shining leaves, such as those of Viscum, Aloe, &c., a very careful examination is necessary to distinguish between the true cuticle and the thickening layer of the epidermis. In the Aloe, the greater part of the so-called cuticle is formed from the cuticular layers of the epidermal cells, and under these layers there lies a real secretion which is the true cuticle. The same may be seen more beautifully in Gasteria obliqua, Viscum, and Phormium tenax. In examining the cuticle, a thin transverse section should be warmed in a solution of caustic potash. The outer covering of such pollen grains as those of the Malvaceæ, which are delicately echinulate, or of the Cichoraceæ, which are rugose, and the outer covering of spores, such as those of Tuber cibarium, must be considered as analogous to the cuticle. Such coverings are completely dissolved by being boiled in a solution of caustic potash, whilst the inner membrane of cellulose remains behind in a free state.

The epidermis covers the leaves and the stems of the higher orders of plants; in the lowest plants, such as the Fungi, Algæ, and Lichens, it is altogether wanting; in the Mosses, it is found in the capsules, in the Marchantiæ on the upper side of the leaf; in Anthoceros it is to be seen on the capsule covered with very beautiful regular stomata. It is present, as has been

already observed, in the higher cryptogams. The young branches of trees are always covered with an epidermis; at a subsequent period a layer of cork is almost always formed underneath it, by which the epidermis is carried off.

The epithelium is an epidermis without stomata: it often consists of papillose cells which frequently secrete a fluid. Epithelium of this nature is seen beautifully in the stigma, in the canal of the style, and in the ovary of phanerogams; the velvety surface of many different kinds of petals, such as the petals of roses, consists of a tissue of this nature. The epidermis of the roots and rootlets, which has no stomata, but which, however, is clothed with hairs, is something of the same nature. Schleiden calls it Epiblema.

The cork consists of many tabular layers formed, for the most part, of thin-walled cells. The layers of cork, when perfect, contain, like the wood vessels, nothing more than air; it is frequently, perhaps periodically, sloughed off with the layer of bark to which it is attached, and it is formed anew from the new layer of bark. It is beautifully developed in some species of Maples, in Ulmus suberosa, in Quercus suber, &c. When the formation of cork takes place in the interior of the bark the layer of cork prevents the flow of sap from the interior, and all the cells exterior to the cork die away. The effect of such a formation of cork may be observed beautifully in Pinus sylvestris, Picea vulgaris, the Oak, and other trees. I draw a distinction between common cork (suber) and leathery cork (periderm); the latter is finely developed in the Birch, and may be seen also in Abies pectinata, in the beech, the alder, the cherry, and generally in all smooth stemmed trees.*

Vascular bundles are formed of cells of different but of specified kinds, united together in groups, the bundles standing in mutual relation to one another, and forming, to a certain extent, a system which pervades the higher orders of plants. Their origin may be best traced in the germination of the seed.

* Upon this point see the chapter on Wood and Bark in the Author's work, "Der Baum."

In the part of the Embryo which forms the stem, they first appear as a cambium bundle immediately underneath the cotyledons.

The elongated thin-walled cells, called the cambium cells, are the most essential part of a vascular bundle. Perfect vascular bundles consisting only of these cells are sometimes, though rarely, met with; in the creeping root and in the runners of Epipogium Gmelini a slight indication of vessels is sometimes, though very rarely, to be seen; these vessels appear first in the stem and in the parts of the flower. In Najas and Caulinia no vessels but only cambium bundles are to be found. Where a new vascular bundle is formed, for instance, in the embryo of phanerogamous plants, it consists, in the first instance, only of cambium cells, some of which, at a subsequent period, become developed into vessels. The position of these cambium cells regulates the growth of the vascular bundle, as well as the nature of the growth of the plant itself. In the dicotyledonous vascular bundle the cambium layer is situated on the outside, that is to say, is turned towards the periphery of the stem; the cambium here meets with no impediment in its outward growth, it is capable of forming new wood on the inside and new bark on the outside, and by this means the stem is enabled to increase in circumference. Schleiden calls this the free vascular bundle, in contradistinction to the closed vascular bundle, that is, the vascular bundle surrounded by wood-cells.

The closed (or definite) vascular bundle is peculiar to monocotyledons and the higher cryptogams; in these the cambium cells are surrounded by thickened cells; the vascular bundle cannot, therefore, increase in circumference.

The growth of the vascular bundle in the stem and in the roots of the higher plants is promoted by a tissue which, in a transverse section of a stem or root, assumes an annular form, and which is to be seen even in the axis of the ripe embryo, where it separates the pith from the bark. This ring, which, in the stem and root, loses itself in the primary parenchyma of the punctum vegetationis, I call the cambium ring, or thickening ring. In this cambium ring the first vascular bundles of the Embryo originate, and by means of it the

circular disposition of the vascular bundles and their growth breadth-wise is produced. The bundles, themselves, increase in length with the growth of the ring. When the ring becomes inactive the growth in thickness ceases also, as is the case with the roots of monocotyledons, where the activity of the ring ceases at an early stage. In dicotyledons the cambium of the vascular bundles coincides with the cambium ring, and each vascular bundle, therefore, progresses in growth on both sides, forming wood and secondary bark. In monocotyledons the vascular bundles ramify in the thickening ring. As the stem therefore increases in thickness, the number of vascular bundles which appear to be divided in a transverse section increases also. As the cambium of the monocotyledonous vascular bundle does not coincide with the cambium ring, it does not grow on both sides, and therefore there is no marked separation of the wood and bark of the vascular bundle, nor is there any special wood-ring. The vascular bundle ramifies in the cambium ring without itself increasing in size. The same circumstances take place in the higher Cryptogamia. The thickening ring itself may be studied in the stem of Isoctes, and its effect upon the growth of the vascular bundle may be observed in Urtica. The ramification of the vascular bundle through the cambium ring is seen in the germination of Palms and other monocotyledons, whilst the germination of trees with acerose or deciduous leaves exhibits the effect of the cambium ring in the formation of wood and secondary bark.*

Besides the cambium cells, which are never wanting, the vascular bundles generally contain *vessels* and *wood-cells*. Vessels are cells placed one above another, whose transverse partition-walls are broken through, and which contain air. The arrangement of the vascular bundles of dicotyledons can only be studied at the apex of a young shoot, or in the embryo, where each newly-formed vascular bundle can be distinguished separately. They may be studied beautifully in Viscum, Tilia, and Pinus. In Palms the cambium layer is situated between the

* *See* "Die Pflanzenzelle," p. 246, and "Der Baum," p. 108, by the Author of this work.

large vessels and the woody fibres, which are generally much developed; in Ferns, it surround the vessels, but is itself surrounded by a more or less strongly developed ring of wood-cells.

Vascular bundles are never formed in the bark; they pass, however, from the stem through the bark into the branches and leaves; if, therefore, a horizontal section be made through the bark, the vascular bundles appear to be cut through in an oblique direction.

The bundles of the liber are those parts of the dicotyledonous vascular bundles which lie in the bark, the woody part of which forms the wood-ring of our trees. In Viscum, liber-cells are found intermixed with woody fibre. The liber bundles in the bark of some Palms are branches of vascular bundles, the cells of which have become developed as liber-cells. Liber bundles, or bundles of a precisely similar nature, are to be found, though less frequently, in the interior of the stems of Palms. In the Apocyneæ and Asclepiadeæ liber-cells are to be found, which contain a milky juice. The so-called laticiferous vessels of the Euphorbiaceæ, Papaveraceæ, &c., are, according to my observations, branched laticiferous liber-cells. Real laticiferous vessels pervading plants in a continuous net-like form, and concerning which so much nonsense has been written, are, as far as my observations extend, very seldom to be met with. The so-called laticiferous vessels of Fungi are cells containing a coloured juice.

At the points where many cells meet there are frequently to be found between the cells, chasms, filled sometimes with air, and sometimes, though less frequently, with fluid. These are called the intercellular spaces; they are to be seen beautifully in a transverse section of the leaf stalk of Cycas revoluta; they are also to be found in most parenchymatal tissue, such as the pith of most trees. These intercellular spaces form, as it were, continuous air-passages surrounding the cells, which air-passages seem to debouch into the breathing pores underneath the stomata.

In addition to the above, we meet with air-passages or air-canals, *i. e.*, larger spaces filled with air which traverse portions

of plants to a greater extent, and which are found especially in water-plants, as in the leaf-stalk of Nymphœa. The cells of plants vary very much, according to their functions. One kind of cell requires different chemical substances from another; one kind of cell effects changes in the same chemical substances different from those produced by another kind of cell; whence arises the different nature of the contents of the cells of one and the same plant. As one cell operates upon another, so one cell affords nourishment to another cell. The whole plant is a compound entity consisting of many cells of unlike properties. The life of the plant can only be learnt by studying the life of its cells.

CHAPTER VII.

CONCERNING THE METHOD OF INVESTIGATION.

The successful result of any inquiry depends, to a very great extent, upon the method of investigation which may be adopted; if the method is accurate, the result will be valuable; if, on the other hand, the method be erroneous, the result will prove nothing. The method is accurate when it is adapted to the question which it is wished to determine, and to the object which is under examination. It is necessary, therefore, that the question should be accurately propounded, and that an accurate use should be made of the proper means for solving the question. In order to be able to propound the question properly, it is necessary to know beforehand why the question is put in one form instead of another, and what it is that the answer will determine; in order to be able to make use of the proper means for solving the question, these means, and the effect to be produced by them, must both be known.

Before entering upon investigation therefore it is necessary to obtain a general acquaintance with the object to be investigated. With regard to philosophical questions which are still matters of controversy, this knowledge only will not be sufficient; in this case it is necessary to be acquainted with the different views which have been taken of the question, and the investigations upon which those views have been founded. Before publishing any philosophical treatise, the writer should not neglect to make himself familiar, as far as possible, with all the recent observations upon the matter in question. By proceeding thus, he will be far less likely to overlook anything of importance, he will obtain more extensive ideas of the subject, his enquiries will be better grounded, he will be able more distinctly to ascertain the value of the opinion which he had

himself formed upon the matter, and will thus arrive at a more certain result. In addition, he will obtain a general historical view of the progress of developement of the question at issue.

The great progress which has been made in natural philosophy in this century is owing, in a great degree, to the adoption of the method of induction, which alone is capable of furthering such progress. Although the method of induction leads from individual to general results, that is to say, from the part to the whole, I should, nevertheless, in microscopical investigations, presuppose a superficial general knowledge of the object to be examined. An accurate investigation of the individual parts of the whole will then lead to an accurate acquaintance with the object in all its particulars; in other words, investigation must begin with generalities, must pass from generalities to details, and lead through the details to an accurate acquaintance with the whole.

It will, perhaps, be objected that a superficial acquaintance with an object is unnecessary for the examination of its details. I believe, however, that although in some cases an accurate acquaintance with the whole may be obtained without such superficial acquaintance in the first instance, nevertheless, the inquirer is far more liable to be deceived, and consumes far more time by proceeding without it. In the inquiries connected with the development of plants, I consider it in many cases impossible to arrive at an accurate result without a superficial acquaintance with all the parts of the plant in a perfect state, inasmuch as, without such knowledge, the observer cannot tell what points to direct his attention to, nor what inquiries he should set on foot. I call the knowledge of the entire perfect plant, which is obtained by the naked eye, or by the help of a magnifying-glass, a superficial knowledge, in opposition to the more accurate knowledge which is obtained by a complete examination, within and without, of the individual parts with different magnifying powers. If, in this latter way, the observer has become acquainted with the individual parts, and their relation to one another, he naturally becomes acquainted with the whole plant, and that, not superficially, as in the first instance, but accurately, both within and without.

The course of investigation to be adopted, is, in its fundamental principles, always the same, but it must, as has been observed, be modified in different ways, according to the sort of question which is required to be solved, and the nature of the object to be examined. The investigation of the outward form will require a different mode of proceeding from that which must be adopted in inquiring into the circumstances relating to structure. The inquiries into the development of different portions of plants must be conducted differently from those connected with the development of the cells. It often happens in the course of an investigation that the inquirer is led aside to a collateral question; it not unfrequently happens that the principal question itself becomes essentially changed during the investigation. The collateral questions generally require a particular answer; the principal question must never be lost sight of in answering the collateral ones; particular care must be taken to endeavour to throw light upon the principal question from all possible sources, for which purpose the collateral questions frequently afford opportunity. In this case they must never be neglected. Where, on the contrary, they have no bearing upon the principal question, it is often better in the first instance to pass them by. In carrying out the investigation, care must be taken to pay attention to every point which can in any way facilitate the solution of the principal question; everything must be most accurately weighed, and examined most fully and scrupulously. By this means a safe result will be obtained. The collateral questions which have no bearing upon the principal question, frequently leave materials for future investigations.

My own experience leads me to think that it is not advisable to be occupied with many investigations at the same time. One complete investigation sufficiently employs the mind and the time of the observer. The work will not be so well performed if it is always being changed. Inquiries relative to the development of a plant, sometimes form an exception to this rule, since it not unfrequently happens that, in order to follow out the successive developements, it is necessary to examine the same object from week to week. In such cases it may be

well in the mean time to carry out some other investigation, but then it is indispensable for the observer to keep written memoranda of his observations, and a note of the date of them, inasmuch as the determination of the length of time within which any particular portion of a plant of which the progressive stages have been watched, comes to perfection, is often a matter of great importance.

From the manifold variety of plants and their different members, it is hardly possible to point out an accurate method of proceeding for all possible cases; the experienced observer will know how to lay out a plan of proceeding suitable to the question proposed, and in accordance with the nature of the object; to the less experienced observer, however, I will give as good advice and assistance as I can. I must here separate the investigation of perfect plants, or of portions of perfect plants, from the inquiries relating to their developement, and I prefer to begin with the former as being the most easy. Both divisions of the subject must be treated from two points of view; from the morphological, which relates to their outward form, and the anatomical, which relates to their internal structure. I recommend everybody who is able to draw, to represent on paper, as accurately as possible, all the objects which, in any microscopical inquiries, may appear to him interesting or important; and to add short notes of everything which cannot be accurately represented by the drawing. Too much cannot be done in this way. In matters relating to morphology, simple and accurate outlines are often quite sufficient; in anatomico-physiological inquiries, on the other hand, every individual cell with its contents must be accurately represented. By a series of such drawings, to which in difficult cases mounted objects must be added, a comparison of the different parts of plants, or of the different conditions of developement of particular portions of plants, is much facilitated: by this means also, the knowledge of them is advanced, and, in many cases, can only thus be obtained.

Accurate drawings should always be made at the time of everything which appears to be important.

If the observer draws with the camera-lucida, and has some

experience in the management of the pencil and brush and in the use of colour, the loss of time will be compensated tenfold by the value of the drawings. Drawings from memory are in all cases to be deprecated, inasmuch as they only afford a representation of the observer's ideas, and not of the object itself; these ideas are *subjective*, and therefore liable to be erroneous.

Besides making drawings and preserving objects, it is a good plan to make notes at the time of everything that appears to be important, and even of matters which may not at the time seem to be of much value, inasmuch as during an investigation it is impossible to tell in many cases what influence small matters may have over the result. It is unsafe, especially in extensive investigations, to rely upon the memory; by so doing many things will be forgotten, and many things insufficiently or inaccurately described. Short notes should always be made, at the latest the same evening, of the things which have been observed during the day, and it is useful to add the date of the observation.

It is indispensable also to preserve a memorandum of the magnifying power employed. In difficult cases a note should be made both of the object-glass and eye-glass employed, inasmuch as it is by no means the same thing whether an observation, in other respects similar as regards magnifying power, was made with a strong object-glass and a low eye-glass, or, on the contrary, with a low object-glass and a powerful eye-glass. An observation with a powerful object-glass and a low eye-glass will always carry far more weight. Low magnifying powers are generally sufficient for purely morphological investigations; in these cases it will frequently be necessary to employ incident light; the preparation of the object will here generally be limited to a separation of its parts; the simple microscope and the needle will have to be used more than the knife for the separation of small parts. It will very seldom happen that an observation of the outer form alone will be satisfactory; it will generally be desirable to inquire also into the internal construction of some one part or another; anatomical investigation must therefore be added to the morphological. For anatomical investigations transmitted light is far more generally employed,

and the use of the knife will be found of great importance; the needle and the simple microscope will only be of service in improving thin sections by the removal of unnecessary portions of them. The use of re-agents will throw a light upon the chemical nature of particular parts.

Since, then, morphological and anatomical investigations go hand in hand, I will treat of them both together. I think it is the best plan to begin with the lower orders of plants, as being the simplest products of the vegetable kingdom, and to pass from them to the investigation of the more highly-developed plants. For the same reason, I should advise the beginner to commence his studies with the lower orders of plants; the minuteness of their parts will prove but little hindrance to him when he has gained some dexterity in preparation with the simple microscope. In investigating the more highly-organized plants far greater difficulties will be met with; difficulties will arise which can only be unravelled by an accurate and general knowledge of their construction.

In entering more in detail into the methods of investigation to be employed, the inquiry into the origin of the plant, in other words, the history of its developement, must be separated from the investigation of the perfect plant. We will begin with the latter.

On the method of examining the Perfect Plant.—Amongst the Cryptogamic plants, the cellular plants, that is to say, those which have no clearly developed vascular bundles, such as Fungi, Algæ, Lichens, Characeæ, Mosses, and Liverworts, are the most simple. In the first four groups, notwithstanding the great variety of form which exists in their individual parts, no division into stem and leaves can be found; real leaves, that is to say, organs which have a different process of developement from that of the stem, appear first in the Mosses and Liverworts. Hardly any preparation is necessary for the examination of the lowest forms of Fungi, such as the floeculent fungi, which is the class to which the different sorts of mould belong; nor is any preparation necessary for the examination of the lowest forms of Algæ, viz., the Confervæ, which consist only of cellular threads. It is sufficient in these cases to disentangle the

twisted threads under the simple mieroseope by the help of a needle, and to elean the plants by rinsing them with water. Partieular attention must be paid to the nature of the eells, both with regard to their walls and their eontents; the use of a solution of iodine, and of iodine and sulphurie aeid, will often be advisable. The eonstruetion of the Charaeeæ also may be studied tolerably well without any speeial preparation; they are frequently encrusted with earbonate of lime, whieh may be removed with very dilute sulphurie aeid.

Cell-division may be observed in some of the Algæ, partieularly in Cladophora and Ulothrix; and in doing this the use of ehemieal re-agents is of great importanee.

In examining the anatomy of the more highly developed Fungi (sueh as the Pileati and Cupulati), or that of the higher orders of Algæ, sueh as the Fueaceæ, or that of Lichens, it is neeessary to take thin sections from different parts of the plants, and in different but definite direetions. Dry Fueaeeæ and Liehens may be very well softened by letting them lie for some hours in eold water; the seetion may be made either with the unassisted hand or between eork. In examining Fungi, fresh speeimens should be used; dry speeimens should never be used for examination when it is possible to obtain fresh plants; in inquiries eonneeted with the developement of plants fresh speeimens are indispensable.

In certain of the Pileate fungi the formation of the spores must be sought for on the under-side of the Pileus, where will be seen the sterigmata, that is to say, stalk-like elongations of the outer extremity of the eells; upon this elongation a spore is formed, which, by the separation of the stalk, beeomes free; eaeh spore-eell or basidium generally produees four of these stalked spores, but there are eertain genera in whieh Basidia are met with bearing only two or even one spore, as for instanee Caloeera viseosa. The spores of the higher Algæ are situated partly upon the surfaee and partly in the hollows of the thallus, and sometimes in peeuliar fruetifying branehes, as in Fueus, where the fruit is developed at the extremities of the thallus, and therefore it must be sought for by making a series of transverse seetions beginning at the extremity, and longitudinal

sections through the middle of the thallus. In lichens the spores are found in peculiar sacs or asci surrounded by paraphyses; the parts of the thallus at which the fructification occurs generally assume the appearance of bowls or cups. As specimens of Lichens, may be mentioned Borrera ciliaris and Peltigera canina, or Peltigera venosa. Fresh specimens only can be used for examining the developement of the spores; a weak solution of iodine renders the asci and paraphyses more or less blue.

There is no broadly defined *anatomical* distinction between Lichens and Fungi, since there are many of the latter which perfect their spores in the interior of spore-tubes (asci), as for instance, Tuber cibarium, Helvella, Morchella, and Peziza.

The tissue of the higher Fungi, as well as of Lichens, consists of threads formed of cells much entangled with one another; even the gonidials cells of Lichens appear to me to be formed of filamentous tissue, the cells, however, being shorter, and still more entangled, as is the case in Calocera viscosa. The spore-cells (*i. e.*, the Asci and Basidia) are the terminal joints of these cellular threads. It is sometimes possible by making delicate sections, and boiling them in water, or treating them with an alkali to detach the individual cellular threads from one another, and to ascertain their mode of attachment *inter se*. The tissue of Fungi is seldom, if ever, turned blue by iodine and sulphuric acid.

In the Fucaceæ, the form and arrangement of the cells vary considerably according to the species of the plant; since elongated cells are to be found in them, it is indispensably necessary that a longitudinal section through the middle of the thallus should be taken in addition to a transverse section. The genus Caulerpa is of a most elegant structure. It consists, of a single cell, which, according to its form, may be looked upon as a stem, leaf, or root. The very thick wall of this single cell sends out branched threads of cellulose into the interior of its hollow. A similar formation is found in the anterior sac-shaped prolongation of the embryo-sac of the half-ripe seed of Pedicularis sylvatica.* In examining this formation it is

* *See* Schacht, "Die Pflanzenzelle," p. 140, Taf. 1, Figs. 7, 8, 10.

necessary to make delicate transverse and longitudinal sections.* The re-agents, such as iodine, the iodized solution of chloride of zinc, and iodine and sulphuric acid, must not be neglected.

The so-called antheridia of the Florideæ and Fucaceæ (divisions of the higher Algæ) are not, in my opinion, of the same nature as the real antheridia of the higher Cryptogams, in which moving spiral threads (antherozoids) are developed. In the above-named Algæ there are certain special cells in which numerous very minute cells are developed, which latter cells become free. Thuret, in the "Annales des Sciences Naturelles," 1854, states that in the Florideæ these latter cells are motionless, and unprovided with cilia, whilst in the Fucaceæ on the other hand, they have motion, and are furnished with a long vibrating cilium and a short motionless one. Similar organisms occurring in the Lichens were also called antheridia by Itzigsohn. I have satisfied myself that the antherozoids which Itzigsohn thought he saw are not present, but there may be small free cells devoid of motion. Tulasne and de Bary have directed attention to certain small cells, unconnected with the formation of real spores, which in many Lichens and Fungi are formed in particular places, and in a particular manner. These small cells, the relation of which to the plant is not yet understood, but which exert no influence upon the germination of the true spores, may perhaps be analogous to the above-mentioned cells of the so-called antheridia of the Florideæ and Fucaceæ. They have been called Spermogonia.†

In the Characeæ, as well as in the following groups of cryptogamous plants, true antheridia are known to exist. In the more highly developed groups, however, such as the Equisetaceæ and the Ferns, the antheridia are not found in the perfect plant, but at the time of germination. The antheridia are of a much more complex form in the Characeæ than in the rest of the cryptogams; the cells in which the spiral filaments are developed are here strung together like a row of pearls, whilst in the antheridia of all other cryptogams they appear

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* *See* "Die Pflanzenzelle," p. 26, Taf. VI., Figs. 1—5, and Fig. 8.

† *See* de Bary "Über die Brandpilze." Berlin, bei G. W. F. Müller, 1853.

separate. The spores also of the Characeæ differ in their position and construction from the spores of all the other Cryptogams; a single large cell, the true spore, is surrounded by a special covering formed of five cells, which answers to the Archegonium or pro-thallus of the Ferns. In the cells of the stems of the Characeæ the motion of the juices of the cell may often be well observed; the species Nitella is the best for this purpose; fresh vigorous plants should be taken during warm weather, and examined as soon as possible.

In the Mosses and Liverworts we first meet with a stem and leaves; both parts must here be particularly noticed. The leaves of the Liverwort always consist of a single layer of cells; the mid-rib, which characterizes the leaves of Mosses, is always wanting. In both these plants it will generally be sufficient to examine the leaves externally, but it is not so with the stem; careful longitudinal and transverse sections must be taken from the stem, either with the unassisted hand (which may be done by a little perseverance), or by the section instrument. It is, moreover, by no means impossible to make thin transverse sections of the leaves. By treating the stem of Cynclidium stygium, or the leafy stem of Diplolæna Lyellii, in this manner, the first indications of a central vascular bundle will be seen, consisting of elongated narrow cells; in Sphagnum, on the other hand, is to be found a concentric ring, consisting of elongated, thickened, brown cells, which, to a certain extent, makes a division between pith and bark, and which is surrounded by large perforated cells, which form, as it were, the bark. In Plagiochila and, as I believe, in all leafy liverworts, as well as in many mosses, the cells of the circumference of the stem are thickened, but every indication of the vascular bundles is wanting. The whole construction of these little plants is far more complicated than that of the before-mentioned groups; this complexity is seen particularly in the construction of the reproductive organs; in these plants we meet with pistillidia, that is, organs in which the young fruit is developed; and we generally find leaves which protect the pistillidia. In Liverworts, the morphology, and the form and arrangement of the leaves with respect to the stem, as well as of the perichætial

leaves and perigone with respect to the fruit, can be best studied with the simple microscope, or upon the stage of the simple microscope by the help of a magnifying-glass. The bent needle, or the knife-shaped needle, is of great service here for separating particular portions of the plants. In the ripe fruit, attention must be paid to the construction of its wall, and to its contents. Thin longitudinal and transverse sections made through the half-ripe fruit of a moss afford a beautiful explanation of the construction of the fruit, of the peristome, of the calyptra, &c. The ripe spores must be examined in the same way as pollen; that is to say, in the dry state, and immersed in water, in oil of lemons, and in concentrated sulphuric acid. In examining the elaters of Liverworts, attention must be paid to the nature of their connexion with the spore-case, and to the arrangement of the single or double spiral band within the cell. The cell is liable to be overlooked on account of the delicacy of its walls. Mosses and Liverworts are furnished with antheridia, and the points for the observer to notice are, what position they occupy on the plants; whether they are situated on the same plants as the pistillidia, or on different plants; what is the time of their appearance, what is their form, whether elongated or round, whether their stalks are long or short, and lastly, whether they are provided with a single outer coat, as is the case with most Mosses and Liverworts, or with a double coat, as in Haplomitrium. If the antheridium is ripe, it generally bursts of itself when placed upon a slide in water; the time in which this takes place varies from five to fifteen minutes. In the Mosses (Polytrichum for instance) the ripeness of the antheridia may be ascertained by the exudation of a milky fluid upon the application of a gentle pressure. The smallest possible quantity of the milky fluid exhibits under the microscope numberless antherozoids in rapid motion. In order to see the spiral filaments properly, it would be best to make use of the strongest object-glass and the lowest eye-glass; the addition of a solution of iodine immediately puts a stop to all motion. The form of the spiral filaments is often best seen after their movements have stopped. It is a good plan also to permit the antherozoids when dispersed throughout the water to

dry slowly upon the glass slide. They should be covered with a thin piece of covering glass, and examined as soon as the water has evaporated. By this means the peculiar cilium, which in Polytrichum, Sphagnum, Pellia, Plagiochila, and Haplomitrium, is single and bears a resemblance to a long whip-lash, becomes very visible. The number of the thicker coils of the antherozoid and its delicate prolongation should then be observed. Thuret states that two vibrating cilia are to be found in Mosses and Liverworts. I find, however, that if the antherozoid is in such a position as to enable the observer to see clearly the transition from the lash-like to the thicker portion, only one long cilium is to be found. The dried antherozoids may be kept for years, if the edge of the glass cover be smeared with gum-mucilage.*

In the Rhizocarpæ (which, according to the recent investigations of Mettenius and Hofmeister certainly belong to the Cryptogamia), as well as in the groups of plants after-mentioned, clearly defined vascular bundles are found, in addition to the leaves and stem. The spores and antheridia appear, in the perfect plant, either separate, or united and enclosed in peculiar protective organs. In Salvinia and Pilularia very good transverse and longitudinal sections of the stem and leaves may be obtained by the help of the section instrument; the protective organs of the spores and antheridia must be cut through with the unassisted hand. The same rule will apply to the spore, which must be placed upon the finger, and treated with the razor in the same manner as is hereafter recommended for the ovule.

In the Lycopodiaceæ, Equisetaceæ, and Pterideæ, the stem, the leaves with the developed stomata, and the organs of fructification, should be particularly examined. It is important to observe the arrangement of the parts of the vascular bundles in the stem and leaves; the direction of the longitudinal section must therefore be regulated by the arrangement of these parts, which can be ascertained by a transverse section. It is also very important to trace out accurately the course of the vascular bundles, and particularly the origin of the new vascular

* *See* "Die Pflanzenzelle," p. 110, Taf. V., Figs. 1—28.

bundles, and their connexion with those already existing. In these three groups, the antheridia are never found on the perfect plant; they have been clearly proved to exist during the germination of the Equisetaceæ and Pterideæ. Mettenius and Hofmeister are of opinion that in Isoetes and Selaginella (Lycopodiaceæ) as well as in the Rhizocarpæ, the antherozoids are formed in the small spores, which in the Rhizocarpæ were formerly considered to be pollen-grains. In Mosses and Liverworts antheridia and pistillidia are found, *i. e.*, organs in which the fruit is developed upon the perfect plant, whilst in the Characeæ the antheridium appears on the perfect plant in company with a germ-organ (the so-called spore) from which the young plant breaks forth. In the Ferns and Equisetaceæ, on the other hand, when the spore germinates, a leafy organ called the prothallus, is produced, upon which antheridia and germ-organs, called ovules, are subsequently produced, and from the latter the young plant springs. Finally, the Lycopodiaceæ and Rhizocarpæ have two kinds of spores, large and small; when the large spore germinates it forms a pro-embryo (or pro-thallus), which, however, remains within the covering of the spore, and upon which germ-organs are produced, which break through the spore. About the same time antherozoids are produced in the small spores, which, in the Rhizocarpæ, are formed in the so-called antheridia. The young plant arises from the pro-embryo. The nature of the antheridia, and especially their relation to the pistillidia, or germ-organs, has not yet been sufficiently explained in any group in which they have hitherto been detected.

The antherozoids of Chara are, according to Thuret, furnished with two long cilia; I am of opinion, however, that there is only one cilium, as in the Mosses and Liverworts. The antherozoids of Ferns and Equisetaceæ have the appearance of spirally twisted bands, which are thickly clothed with vibratory cilia either throughout their whole length, or over a certain number of the coils. The antherozoids of Lycopodiaceæ and Rhizocarpæ, with the exception of Isoetes, are, according to Hofmeister, not provided with cilia, but are formed like those of the Mosses and Liverworts. The process of gradual siccation

alluded to above should be adopted with the antherozoids just mentioned.*

In the Lycopodiaccæ the fruit is found in the axils of the leaves, frequently upon branches specially formed for the purpose; in the Equisetaceæ the fruit is collected in ears on the under-side of certain protecting scales similar to the anthers of some of the Coniferæ. The ferns in some instances (such as Pteris, Aspidium, &c.) are provided with stalked thecæ, which are collected in small heaps near one another, generally on the under-side of the leaves, and which burst open when ripe; in other cases, such as Botrychium and Osmunda, the spores are developed in sessile leathery capsules, placed on peculiar fruit-leaves. I recommend the use of concentrated sulphuric acid in examining spores in general, and particularly in examining the spores and thecæ of the last-named groups. By using this, the number and nature of the coats of the spores may be observed. Schultz's method of maceration is peculiarly well adapted for obtaining an accurate acquaintance with the cells of stems and leaves.

In phanerogamous plants, the axis (*i. e.*, the stem, branches, and roots), and the leaves must be separately considered, and examined in a particular manner. In the higher Cryptogams also, these three essential parts of the plant must be particularly considered. In the Algæ, Lichens, Fungi, and Characeæ, no division into stem leaves and root is to be found; in the Mosses and Liverworts, on the other hand, stem and leaves are always present, but there is no true root. The rest of the Cryptogamia are furnished with all three parts; the toothed sheaths of the joints of the stems of Equisetaceæ form a circle of small leaves united at the base. Mosses and Liverworts have either no vascular bundles at all, or the latter are very slightly developed, as in the cambium bundle of the middle nerve of Diplolæna; whilst in the higher Cryptogamia, they appear in a more or less fully developed condition.

The Examination of the Stem and Root.—In examining the monocotyledonous and cryptogamous stem, particular attention

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* *See* Hofmeister's "Keimung, &c. höherer Kryptogamen."

must be paid to the arrangement of the parts of the vascular bundles, and to the position of these bundles *inter se;* for which purpose a very thin transverse section must first be made. When, by means of that section, the observer has satisfied himself as to the distribution of the dispersed and definite vascular bundles, and as to the position *inter se* of the essential parts of the vascular bundles themselves, he must make thin longitudinal sections in different, but definite, directions through the vascular bundle, in order to obtain a clear idea of the nature of its elements. Attention must first be paid to the cambial cells of the vascular bundles, then to the nature of the vessels, and finally to the ligneous cells of each bundle. In monocotyledonous stems it should further be observed whether a bark can be distinguished, as is the case with palms. If this be so, particular attention must be paid to the parenchyma between the vascular bundles, in order to ascertain whether, from the nature of the cells of this parenchyma, it is possible to distinguish a state of tissue intermediate between the bark above mentioned and the proper woody fibre. For an investigation of this nature, fresh specimens are indispensable. The cryptogamous vascular bundle is generally distinguishable from the monocotyledonous vascular bundle by the position of the vessels in the middle of the bundle, as in the Ferns, Lycopodiaceæ, Rhizocarpæ, &c., where the vessels are surrounded by the cambium. The monocotyledonous vascular bundle, on the other hand, always has its Cambium in the middle, surrounded by vessels and wood-cells. In the Palms, a group of very woody cells is generally turned towards the bark, which latter cells may be compared to the bast-cells of the dicotyledonous vascular bundle.

In the vascular bundles of Epipogium Gmelini, and Goodyera repens, which when a transverse section of the stem is made at certain heights appear dispersed in all directions, I can certainly trace a branching, nay more, a regular progression of whole bundles through successive ramifications out of a single central vascular bundle of the Rhizome. In some palms which I examined (Rhapis flabelliformis) I found the vascular bundles divided underneath the summit of the axis. In the embryo of the seed of the date the vascular bundles of the cotyledon are

branched; the place of formation of the vascular bundles lies underneath the plumule. Accurate examination of the process of germination shows that the same is the case with other monocotyledonous plants. An accurate investigation of the course of the monocotyledonous vascular bundle is very much to be desired for the sake of science in general. Many methods may be adopted for this purpose: 1st., If the stem is rotten, the vascular bundles may be laid bare if they are separated by thin-walled parenchyma. 2dly., Where they are surrounded by wood parenchyma, this tissue must be carefully removed with a sharp-pointed scalpel or penknife, and the course of one or more of the vascular bundles be followed out. 3dly., The increase and change of position of the vascular bundles may be shown to exist by means of transverse sections, made at different heights, proceeding from the root or from the lower part of the plant upwards; and then, by means of corresponding longitudinal sections, the mode of this increase of the vascular bundles must be sought after. 4thly., An accurate examination of the course of the vascular bundle in the embryo of the ripe seed is highly to be recommended. I have tried all the above-mentioned four methods; the method of separation from a rotten stem is very apt to lead to deception, inasmuch as the young vascular bundles which have not yet become woody, decay with the thin-walled parenchyma, leaving behind only the older woody bundles. In this case, therefore, portions of the plant are sometimes found detached from one another, which, during the life of the plant were connected together. The second method, in conjunction with the third, leads to much more satisfactory results, but the second method is frequently not practicable. The fourth method, together with the third, removes all possible doubt as to the connexion of the vascular bundles in the interior of the plant. With regard to the third method it will be a good plan accurately to observe and make notes of the distances of the transverse sections from one another. The epidermis of monocotyledonous stems must also be attended to. In some Palms and in Dracæna there is to be seen a layer of cork, more or less highly developed, which originates beneath the epidermis.

The roots of monocotyledonous plants, as far as my observations go, always have a single central vascular bundle, or rather a crown of vascular bundles, which, in the root of sarsaparilla and in the roots of palms, &c., is separated from the outer layer, which may be called bark, by a row of very thick, and generally very narrow, cells. In the arrangement of the parts of this central vascular bundle may be seen, sometimes very clearly, and, indeed, through the separated cambium-groups, the individual vascular bundles which together form the crown of vascular bundles, as in the lateral roots of Cephalanthera and Epipactis.* The root is clothed on the outside with an epidermis which never has stomata, but which frequently sends out long hairs. The examination of the root is conducted in the same way as that of the stem. The extremity of *all* roots is clothed with a hood or cap consisting of dead cells, which cap is in immediate connexion with the growing apex or punctum vegetationis of the root. The longitudinal section must, therefore, pass accurately through the middle of the apex of the root, and it must be extremely fine, so that the connexion between the cap and the punctum vegetationis of the root may be clearly visible. In the ripe embryo of trees with acerose leaves, and in the roots of these trees generally, the root-cap is remarkably developed. In other plants it is only very slightly developed, but it is never altogether wanting.

In the dicotyledonous stem also a transverse section must first be made; this is done by the help of a very sharp razor, either with the unassisted hand, or, if the fragment is small, then with the section instrument. The transverse section must be very thin; the first thing to be looked to is the arrangement of the parts of the stem from the interior to the exterior, which may be divided into four parts: 1st. The Pith; 2nd. The Wood; 3rd. The Cambium; and 4th. The primary and secondary Bark.

1st. In the case of the Pith, the size and form of it, the nature of the cells, the transition from pith-cells to wood-cells, and, lastly, the contents of the pith-cells must be observed. In

* *See* "Die Pflanzenzelle," Taf. XV., Figs 12, 13.

some tropical twining plants, and in the stem and branches of the oak, the chesnut, &c., the pith is not round, but has an angular form.

2nd. As to the Wood. This surrounds the pith, and in it notice must be taken of the arrangement of the medullary rays, that is, those cells which pass in a radiant manner from the pith to the bark; it must be ascertained whether they appear in single rows or in many rows, whether they extend collectively as far as the pith (as is the case in all young plants), or whether some of them being, as it were, secondary (*i.e.*, subsequently developed) rays, are lost in the wood-circle; whether they are numerous and near one another, or fewer in number and at a distance from one another; whether they are all of equal breadth, as in the lime, the willow, the poplar, and in *all* the Coniferæ, or whether both broad and narrow medullary rays are to be found, as is the case in the oak and the beech; and lastly, what becomes of the medullary rays when they reach the bark. The arrangement of the wood-cells must also be observed, that is, whether they are intermixed with vessels, or whether real vessels are wanting, as is the case with the Coniferæ and Cycadeæ. In the Coniferæ particular attention must be paid to the position of the pits, so as to see whether they are only to be met with in the direction of the medullary rays, or whether they are also to be found (which is less frequently the case) in the opposite direction. It must, moreover, be ascertained whether the stem contains turpentine canals; and the position of these canals on the inner side of the annual ring must be noticed. In angiospermous plants the arrangement, size, and mode of thickening of the vessels and the distribution of the surrounding wood-cells is of importance. In all dicotyledonous stems notice must also be taken of the limits of the annual rings, and it must be seen whether they are strongly or slightly developed, or whether they are wanting altogether, as is the case with most tropical trees.

3rd. The Cambium. In observing this, particular attention must be paid to its connexion with the wood on one side, and with the bark on the other. The transverse section must be thin and cleanly made, so that the number of rows of cambium-

cells, and their nature and contents, may be clearly observed; weak alkaline ley is frequently effectual in removing the granular contents, and rendering the cells more transparent; and the observer is then enabled to distinguish the part of the cambium which forms the wood and the vessels from the part which produces the medullary rays. The contents of these cells must first be tested with a solution of iodine, and with sugar and sulphuric acid.

4th. The Bark. In examining this, the attention must first be directed to the presence and arrangement of the liber-cells in the secondary bark, *i. e.*, the bark last formed by the cambium. The primary bark is found even in the embryo and in young buds; the cambium-ring, or thickening-ring, in which, at a later period, the vascular bundles originate, divides this primary bark from the pith. The secondary bark, on the other hand, is first formed by the cambium; it grows together with the wood-ring, and in it is situated the liber of the dicotyledonous vascular bundle; in the primary bark liber-bundles are never found, although, even in it, elongated, strongly-thickened cells, similar to liber-cells, are occasionally met with, as is the case in Casuarina. It is necessary to observe whether the liber appears to be arranged in bundles or in rows, as is the case with the Cupressinæ, and also whether the epidermis, which is never wanting in the young state of a plant, is still to be seen; and whether there is any layer of cork, and what is the extent of it. Attention must also be directed to the nature of the cork, that is, whether a smooth leathery cork (Periderma) envelopes the stem, as in the birch and in Abies pectinata, or whether a chinky, spongy cork (suber) be present, as in the cork-oak and in Acer campestre. Lastly, it is necessary to see whether there is a corky formation in the interior of the bark,* in what manner it is constituted, and how it is cast off. In the Coniferæ it is also necessary to ascertain whether any turpentine canals are to be found.

Besides the above-mentioned transverse sections, longitudinal

* *See*, upon this subject, Hanstein "Über den Bau und die Entwickelung der Baumrinde," and Schacht, "Der Baum."

sections of two different kinds are necessary in examining dicotyledonous stems. The first is a longitudinal section parallel with the medullary rays, and which may be called a radial section. This section must pass from the pith through the wood, the cambium, and the bark. It is only in very thin stems or branches that it is possible to obtain a perfect section of this nature; the difficulty of getting a perfect section is such that it is generally necessary to put up with many different sections, of which one may exhibit the pith and the heart-wood, *i. e.*, the oldest wood surrounding the pith; a second may perhaps show the middle of the wood, and a third the outer part of the wood as well as the cambium and the bark. The same difficulty occurs in making a transverse section of a large stem.

The second kind of longitudinal section which it is necessary to make is one at right angles to the medullary rays, which may be called a tangential section. A section of this nature about the middle of the wood and another through the secondary bark will generally be satisfactory.

In examining the radial section the same course must be pursued as with the transverse section, that is, the attention must be directed separately to the Pith, the Wood, the Cambium, and the Bark.

The Pith.—In this the length and contents of the cells as well as the porous nature of their walls must be noticed.

Secondly, the Wood.—In examining this it must be observed whether the medullary rays are long or short, narrow or wide, whether the pores are large or small, whether any pits are to be seen, and what may be the contents of the cells. Attention must also be paid to the wood-cells and to the presence of a wood-parenchyma, which is very beautifully developed in the Leguminosæ. This parenchyma frequently produces starch, which is never present in true wood-cells. The size and position of the pits, and the form and arrangement of their pores, must also be noticed, as well as the existence of a more or less clearly developed spiral in the wood-cells. This latter occurs in the Yew, the Vine, and in the wood-parenchyma of Ulex and Spartium. It must be seen whether the partition-walls of the cells, by the absorption of which vessels are formed, impinge

upon one another directly or in an oblique direction; in the former case they will be pierced with a round hole, in the latter the partition-walls will be divided in a scalariform manner, as is the case with Alnus, Betula, Corylus, Viburnum, Buxus, and Thea. These two forms seldom occur together in the same stem. Moreover, the nature of the thickening of the vessels must be considered; *i. e.*, whether they appear to be spiral or scalariform vessels; whether they bear pits; and whether the pits and spirals occur simultaneously, as is the case in Tilia, Prunus Padus, Carpinus and Acer. In the Coniferæ, inquiry should be made as to the existence of turpentine canals, and also as to the cellular threads pointed out by Hartig, which are isolated, usually narrow cells, with partition-walls impinging directly upon one another, which contain resin and correspond to the woody-parenchyma of deciduous trees; these latter are found in Thuja, Cupressus, Taxodium, Juniperus, Chamæcyparis, Pinus, and Cedrus, but they appear to be wanting in every instance in which turpentine canals exist in the wood.

Thirdly, the Cambium.—In examining this the form and contents of its cells must be considered, and its gradual transition on the one side into the wood, and on the other into the bark. The cambium when fresh is rich in nitrogenous substances; sugar and sulphuric acid impart a rose-colour to it.

Fourthly and Lastly, the Bark.—In examining this, attention must be paid to the parenchyma and its contents, to the shortness or length of the liber-cells, and also to the secondary liber-cells, which in Abies pectinata are branched, and in Picea vulgaris are cubical and in rows; the lamination of the liber-cells themselves should also be observed. The construction of the cork cells should also be examined if a peridermal layer or a formation such as that mentioned at p. 108 be present.

The tangential section is especially important for the examination of the wood, and of the arrangement of the medullary rays. It may be ascertained by the help of this section, whether the medullary rays form, in the direction of their length, a single row of cells, or whether, in their middle, they consist of more than one row or of numerous rows of cells, in which case they would appear in the transverse section to be

ventricose in the middle and pointed at both ends. This is the case with Laurus Sassafras and Hernandia sonora, and more or less with all the Leguminosæ and with those dicotyledonous woods which have both wide and narrow medullary rays, such as the Oak and the Beech. The medullary rays of Ephedra form two or three rows of cells. When the medullary rays are ventricose, the course of the wood-cells is necessarily a tortuous one. In the Coniferæ, the length of the medullary rays must also be observed. The Juniper has medullary rays, consisting of from one to five, and the Yew of from two to twenty-four cells. In the Coniferæ, there are sometimes to be found horizontal turpentine canals. They occur in the interior of certain of the medullary rays, which are of greater width than the other medullary rays, and are sparingly distributed. These canals are to be found in Pinus sylvestris and Pinus maritima.

The tangential section is important in the case of the Coniferæ for showing the construction of the pits. The lenticular space, and the canaliculus which runs from each of the neighbouring cells into this space, may here be observed. The Yew and Pinus maritima afford excellent examples.

For preparing the specimens, the rules given in another part of this work will apply. With regard to the Coniferæ, and all resinous plants generally, it is a good plan to moisten the surface from which the section is to be taken with alcohol instead of water, and it will be advantageous to lay the section in alcohol before making any observations upon it, partly for the purpose of driving out the air, and partly for dissolving the resin. In the case of the Coniferæ, this soaking in alcohol is indispensable. If it is wished to study more minutely the structure of the individual cells of the stem, I recommend Schultz's method of maceration, and the boiling of delicate transverse and longitudinal sections in alkaline ley, such processes to be followed by the application of a solution of chloride of zinc and iodide of potassium. Very hard woods, such as the woods of tree ferns and palms, may advantageously be laid in water for from twenty-four to forty-eight hours. By so doing, the wood-cells seem to become more tender, and may be cut more easily. The transverse sections of some very hard woods, if

made very thin, always roll up. In this case nothing can be done beyond drawing the parts from one another with a needle, and pressing them flat under a tolerably thick covering glass. Thin sections of soft woods often fold together, in which case they must be placed under the simple microscope and spread out by means of a needle.

The same course may be pursued in examining the roots of dicotyledonous plants as has been recommended for the stem. The root, like the stem, is generally furnished with pith, but this pith is sometimes very small, and, moreover, woody; it is, therefore, occasionally not perceptible. The mode of development of the wood-ring of the root shows, however, that it is always present. In the root the attention should be directed to the extent of development of the root-cap, which in trees with acerose leaves is very highly developed. Perfectly fresh specimens are indispensable for this investigation. Two layers may often be distinguished in the primary bark of the root, of which the outer layer perishes earlier than the inner one. The cells generally of the root are wider than those of the stem; the wood-cells in the roots of trees with acerose leaves have on that account two or three rows of dots, whilst the cells of the stem have only one row.

In examining fossil woods it is sometimes useful to digest them for several days in a solution of carbonate of soda, and then to wash them clean with water. By this means very good sections may generally be obtained of wood, which, without such treatment, would not be manageable. Very good sections of wood which has been impregnated with carbonate of lime may be obtained by using a watch-spring-saw, and afterwards polishing the sections.

The best plan is to make an even section with the saw, to polish the section upon a fine grind-stone with water, and then to use the saw again. A tolerably thin section (longitudinal or transverse) having been thus obtained, it should be fastened with sealing-wax by its polished side to a cork; the coarse parts of it should then be removed with a file, and the section should lastly be ground completely fine upon a grind-stone under water. The cork with the section should then be laid in

alcohol, by which means the section is detached. The section must be cleaned with a camel's-hair brush, and mounted in copal varnish, or Canada balsam. This process may also be adopted in making sections of bone or teeth. With regard to petrified wood, the only plan which can be pursued with advantage is to break off small lamellæ by careful strokes with a steel hammer; the processes of sawing and polishing are generally too tedious for this sort of wood, and seldom lead to a satisfactory result.

The examination of Leaves.—In examining leaves, the first thing that is necessary is to make thin longitudinal and transverse sections through the leaf. If the leaf is not very fleshy the sections are best made by the aid of the section instrument. In examining the epidermis of the leaves of the species Aloe and Agave, and of all other very fleshy leaves, it is necessary to detach the epidermis, together with some of the subjacent cellular layers, and to place it in the section instrument, since there is no other way in which a sufficiently thin section can be obtained.

The first thing to be done is to examine the epidermis, and to ascertain whether both sides of the leaf have the same sort of epidermis, and whether or not it is furnished with stomata; the construction of the stomata themselves, as well as their mode of arrangement, may be learnt by the help of the transverse section, and by examining the detached epidermis from above. With respect to the stomata, their position and arrangement must be observed, and it must be ascertained whether they are spread over the whole surface of the epidermis or are only to be found upon certain parts of it; whether the arrangement of them is regular or irregular; and whether they are on a level with the epidermis, or raised above it, or sunk below it. The nature of the cuticle is learnt by taking very thin transverse sections and treating them with the iodized solution of chloride of zinc, or concentrated sulphuric acid, by boiling with caustic potash, or by maceration in the manner proposed by Schultz. By proceeding thus, it will be seen that that which most authors call the cuticle, embraces two things; that it consists on the outside of a structureless secretion from the epidermal cells, and on the inside of the outer layers of the

epidermal cells themselves chemically altered. These two parts are generally so closely united that they cannot be separated from one another by concentrated sulphuric acid and maceration; by boiling with caustic potash, however, the individual epidermal cells in the plants Gasteria obliqua, Phormium tenax, and Viscum album, become separated from one another, whilst the secretion from the epidermis, *i. e.*, the proper cuticle, is dissolved into a granular matter. A comparison of young and old leaves is here very useful. The hairs which clothe the epidermis, and their mode of insertion and construction, must also be observed, and the arrangement of the parenchyma of the leaf, and the distribution of the vascular bundles in the form of nerves, are also of importance. It very seldom happens (as in Viscum) that the parenchyma of the upper-side of the leaf corresponds altogether in its arrangement with that of the underside; it often happens that one side has air-holes and the other none. In the leaf of many of the Urticeæ and Moreæ peculiar bodies are met with in the interior of certain of the cells. These bodies are pendant, and are attached to small stalks formed of cellulose; they consist of layers of cellulose, and are impregnated with carbonate of lime. They are found in Ficus elastica, Ficus australis, Ficus ulmifolia, Urtica nivea, Cannabis sativa, Humulus lupulus, &c. Similar bodies are found also in the leaves and stems of many Acanthaceæ, such as Justicia, Ruellia, and Barleria.

Beautifully perfect Crystals are found in the leaves of some species of Citrons.

The contents of the cells of the parenchyma and of the epidermis also deserve to be noticed. In the examination of the leaf-stalk, transverse sections must be made at different heights, by means of which the position of the vascular bundles which pass from the stem into the leaf must be observed. By this means it is seen how the side veins of the leaf originate in a continual division of the vascular bundles of the leaf-stalk and mid-rib. Very delicate petals may be advantageously cut with the section instrument, by the aid of which I have not unfrequently succeeded in obtaining very thin transverse sections of the leaves of liverworts, which consist of only one layer of cells.

The cork which is used for delicate petals must be very soft, and the pressure exerted upon the petal must not be too severe.

The examination of the Flower and Fruit.—In examining the flower, the first thing to be observed is the number and position *inter se* of its parts; and afterwards, the construction of these parts themselves must be inquired into. For ascertaining the number and position of the parts of the flower, it is best to take moderately thin transverse sections at different heights through an unopened bud. Such a section from the summit of the bud will generally only show the relative positions of the calyx and the petals, and their situation in the bud. A transverse section made somewhat lower down will, in the case of hermaphrodite flowers, exhibit also the anthers and their relation to the petals, and frequently also the style or stigma. In plants with superior ovaries this section will also show the relation of the stigma to the surrounding parts of the flower. A transverse section made still lower down will generally be required. In flowers with inferior ovaries it is necessary to make transverse sections at different heights through such ovaries. By means of such transverse sections, which must not be too thin, lest the individual parts should fall to pieces, the plan of the flower is seen, and a knowledge of the arrangement of its part is most easily obtained; the different whorls of leaves may thus be clearly observed, and it may be seen how the calyx and the petals are arranged in the bud, what the anthers are like before bursting, whether the sepals and petals and the filaments alternate with one another or not, and moreover, the relation of the divisions of the ovary to the preceding whorl of leaves may also be observed, &c. In transverse sections of this nature, particular care must be taken not to displace any portion of the section by touching it with the needle or any other instrument. In young buds this may easily be avoided by a little care; but buds which are on the point of opening cannot be used for making transverse sections. These sections, and indeed all sorts of sections, are removed from the knife with a fine camel's-hair brush. Sections which are not made quite horizontally through the bud are of no value.

Besides the above-mentioned transverse sections, which are

of great importance for an exact analysis of the flower, it is also necessary to make longitudinal sections exactly through the middle of the buds, in the directions shown to be necessary by the transverse section. By means of these longitudinal sections, the insertion of the petals and stamens may most easily be observed; and it may also be ascertained whether they originate from nearly the same point as the sepals, or whether they are borne upon a disc; it may also be seen whether in monopetalous corollas the filaments of the anthers are joined to the corolla, and at what point they separate from it. It may also be observed what is the position of the ovary with respect to the other parts of the flower; whether it is superior or inferior or intermediate between the two, in what manner the style is united to the ovary, and how the canal of the style is connected with the partitions of the ovary. In many cases these questions can only be decided by observing the developement of the ovary and the style. The transverse and longitudinal sections of the bud afford considerable information concerning the nature of the hairs which clothe the blossom. In the Compositæ, a longitudinal section through the whole capitulum must be taken, in addition to the sections through the individual blossoms.

When by these means a general knowledge has been obtained of the relation of the parts to one another, the observer should pull to pieces some buds which are on the point of opening, in order to obtain a more intimate acquaintance with the individual parts of the flower, which he must then proceed to examine.

All the above general directions with respect to the anatomical examination of the leaves will apply to the bract and the calyx. In making an analysis of the flower, its exterior must first be observed, that is to say, its form and colour, and the nature of the hairs with which it is covered; it must then be ascertained whether the tissue is succulent, woody, leathery, or dry; and the changes which take place in the tissue after the period of blossoming must also be noticed.

There is but little to be said with respect to the petals. By the aid of thin transverse and longitudinal sections, made with the section instrument, the construction of the petals and of their

epidermis may be ascertained. By examining the whole surface of the petals with a low magnifying power, or with incident light, the distribution of its vascular bundles is made clear. It is these bundles which frequently produce the delicate lines upon the petals. The fluid contents of the cells, which in the petals are frequently of such beautiful colours, must be particularly noticed. The form of the petals, their colour, and the nature of their exterior, are important in analysing the flower. In dealing with the stamens, the anthers must be particularly attended to; they must be examined out of the bud before the latter has opened, and also shortly before and after dehiscence; in the latter case a transverse section is seldom practicable. The anthers, whilst in the bud, will generally be found to be quadrilocular. The cellular mass which divides the two loculi of each side is afterwards either wholly or partly absorbed, so that, at the time of the flower opening, the anther appears to be bilocular. Bilocular anthers are, however, also to be met with. The anthers of many acerose-leaved trees are bilocular, as of Abies, Picea, Pinus, and Larix, whilst those of Cupressus and Taxus, and of the Cycadeæ, are furnished with many single loculi; bilocular anthers are also to be found amongst the Amaranthaceæ, in Gomphrena decumbens, and Alternanthera diffusa. Albersia and Celosia, however, have normally quadrilocular anthers. The anther of Meriolix serrulata develops its pollen in detached groups from mother-cells; it is not until some time afterwards that the parenchyma which divided these groups disappears; the anther opens, as in the rest of the Onagraceæ, with two longitudinal fissures. In Viscum, also, the mother-cells appear in groups, separated from one another by parenchyma. Since these circumstances cannot always be predicated with certainty, it is indispensable for an accurate analysis of a flower, that a transverse section be taken through the anther whilst in the bud. The connective of the anther, which is always characterized by its vascular bundle, must also be examined, as well as the loculi and their walls, and particular attention must be paid to certain cells which are generally to be found in the walls of anthers, and which contain a delicate spiral band.

The position of these cells, *i. e.*, whether they form the outer or the inner layer, should also be noticed. In Abies and Picea, these spiral cells form the outer layer of the wall of the anther; in Quercus, Fagus, Hippuris, and the greater number of plants, they appear in the form of a second (the innermost) layer. In Monotropa, the spiral thickening of these cells is wanting. In all the anthers which I have examined, the wall in its perfect state always consists of only two layers of cells.

In considering the anthers morphologically, we must observe the nature of their attachment to the filament and their form, whether divided or otherwise; in Betula, Alnus, Corylus, and Carpinus, the filament is furcated, and each part bears a bilocular anther. We must also observe their mode of dehiscence, and the form of their valves. It must be seen whether they are provided with elongated processes at either end (which may be well observed in the Compositæ), and whether the loculi are fully developed on both sides of the connective, or whether only one loculus produces pollen, as is the case in Salvia. The form, also, of the filaments, must be accurately observed, so as to ascertain whether they are short or long, straight or bent, simple or divided, or furnished with appendicular processes. The latter is the case with Asclepias and Borago. The insertion of the filaments must also be looked to, and it must be seen whether they appear to be united to one another (as in Ruscus and some of the Amaranthaceæ), and what is the nature of the connexion, if any.

The contents of the anthers, *i. e.*, the ripe pollen, must be carefully considered. It is examined dry, and also in water, in oil of lemons, and in concentrated sulphuric acid; in some cases it will also be judicious to treat it with the iodized solution of chloride of zinc and with nitric acid. In examining the pollen particular attention must be paid to the structure of its coatings, and to the places destined for the egress of the pollen-tubes. The number and disposition of these openings must be considered, as well as the questions whether they lie in depressions of the outer coating of the pollen (which can frequently only be ascertained by treating the pollen under water), or whether they are provided with opercula, as is the case with the Stellaria.

The construction of the outer coating, the cuticle, which frequently assumes the most delicate forms (the beauty of which may be seen in the Cichoraceæ, in Stellaria, the Cucurbitaceæ, the Passifloreæ, and Amaranthaeeæ) merits attention, and the colour of this cuticle when treated with sulphuric acid should be particularly observed. In treating the pollen-masses of the Asclepiadeæ, thin sections should be taken by means of the section instrument. The leathery outer layer, which turns red upon the application of concentrated sulphuric acid, will then be seen to be a secretion from the pollen-cells. In orchideous plants, the connexion of the pollen-masses with the caudiculus and the retinaculum must be observed; each pollen-mass must first be examined separately, and afterwards the individual grains of pollen, which latter must be submitted to different tests. It will frequently be well worth while to observe the egress of the pollen-tube, especially when the pollen appears to have only one coat; it is generally easy to obtain pollen tubes by applying the pollen to the stigma; they generally appear in numbers after a time, varying from one to eight days. The moisture which is secreted from the stigma of the flowers of Hoya carnosa is well adapted for the formation of pollen-tubes; if the pollen of other plants is brought to their style, very beautiful pollen-tubes are generally obtained. The use of syrup is seldom attended with any success. Fresh pollen is always preferable for examination to that which has become dry. Although the cuticle is not injured, the contents of the pollen-grains often become changed by keeping. In the fresh pollen of accrose-leaved trees a small body consisting of many cells is to be seen in the interior of the special pollen-cell, which body adheres firmly to the wall of the pollen-cell. If nitric acid be used, the pollen-cell and the small body make their way out of the cuticle. The body cannot be discovered in old pollen-grains.*

We will now consider the style and stigma. These may exist singly or in greater numbers. Thin longitudinal sections are generally sufficient. In the stigma, attention must first be paid to the tissue which secretes a fluid matter, and which is generally

*. *See* Schacht's "Beiträge zur Anatomie und Physiologie der Gewächse," p. 148.

covered with papillæ. In the style, the most important points to be attended to are, the course of the canal and the nature of its conducting tissue. A thin transverse section through the style is often useful; by means of it the distribution of the vascular bundles may be ascertained.

In examining the ovary it is necessary to make very thin transverse sections at different heights. If the ovary appears to be multilocular, it will sometimes be necessary to have recourse to the needle and the simple microscope to determine whether such is really the case. Very many ovaries which are stated in books to be multilocular with a central placenta, are, in reality, at least in the upper part, unilocular ovaries with many parietal placentæ lying close to one another, being multilocular, however, in the lower part. This is the case in the Onagrariæ, Pyrolaceæ, and Monotropeæ. The ovary of the Cucurbitaceæ, on the other hand, is unilocular throughout its entire length. In the Onagrariæ there are four parietal placentæ which, in a transverse section, project like a border into the hollow of the ovary. They spread out at their extremities on both sides, bear an ovule on each side, and lie close to one another. Between these four placentæ, which are in contact with one another, there is formed an open space, which serves to a certain extent, as a prolongation of the canal of the style; the four placentæ are united at the lower part of the ovary. In examining the transverse sections of the ovary, the attention must also be directed to the arrangement of the placentæ, and to the distribution of the ovules upon the latter. The distribution of the vascular bundles in the ovary and in the placentæ should also be observed, as well as the hairs which clothe the ovary.*

The longitudinal section through the middle of the ovary is regulated, partly by the arrangement of the placentæ, and partly by the position of the style and stigma. It will frequently be necessary to make longitudinal sections in different directions through the middle of the ovary, and, if possible, also through the middle of the style, and through part of the stigma. It

* *See* Schacht's "Beiträge," &c., Chap. VI.

will frequently be neeessary to make longitudinal sections in different directions through the middle of the ovary, and, if possible, also through the middle of the style, and through part of the stigma. It will more frequently happen that from the impossibility of making such longitudinal sections the stigma and style must be examined separately. In examining longitudinal sections of the ovary the attention must again be directed to the placentæ and ovules, to the position of the latter, to the connexion of the canal of the style with the hollow of the ovary, to the distribution of the vascular bundles which pass from the peduncle into the ovary, and to the subsequent ramifications of these bundles in the other parts of the flower.

The most important part of the ovary is the ovule, the condition of which at the period of blossoming must be considered. Three things must be attended to in considering the ovule. 1st. The existence and the number of the coats of the ovule; 2ndly. The position of the ovule, and especially the situation of the micropyle with regard to the hilum; 3rdly. The situation of the embryo-sac, and its relation to the nucleus.

These questions can seldom be solved by examining the whole ovule; in the Orchideæ, in Monotropa, and in certain species of Pyrola, the ovules of which are very small and transparent, and whose delicate nature precludes the possibility of preparing them in any way, it is possible, by accurate adjustment, to examine the ovules entire. In most cases, however, thin longitudinal sections must be made exactly through the middle of the ovule; in particular cases, such as in Œnothera, the best way of doing this is to make thin longitudinal sections through the ovary itself; amongst the many ovules which will be thus cut through, some will be found here and there to have been accurately divided; these must be extracted under the simple microscope. In other plants, such as Iris and Cucurbita, a transverse section is more advantageous. In almost all other cases it will be necessary to detach the ovule itself, to place it upon the forefinger, and to make two cuts through it with a very sharp razor, so as to obtain a thin longitudinal lamella forming exactly the middle of the ovule. The best way of doing this is to slice off one side of the ovule, to turn the ovule round

with a fine camel's-hair brush, and then to slice off the other side of the ovule. The section thus obtained must be placed under the microscope; if it be only a moderately good one, it may frequently be improved by a third or fourth cut made in the same manner. The correct position of the ovule upon the finger must first be ascertained by the help of a lens. The ovules of all the Personatæ, of the Labiatæ, of the Borragineæ, of the Coniferæ, &c., require to be treated in this manner. In particular cases the existence of the coats of the ovule cannot be clearly ascertained even with the most successful sections—this is particularly the case where the nucleus is very slightly developed and very soon displaced by the embryo sac. In this case, unless the nature of the developement is known, it must be doubtful whether a naked nucleus or a single highly-developed integument is present. As an instance of this, I may mention Asclepias Syriaca, the developement of which will be fully considered hereafter.

With respect to the position of the ovule, three principal types may be mentioned. 1st. The orthotropal ovule, where the micropyle lies in a direct line over the hilum, as in Hydrocharis, Taxus, Juglans and Polygonum; 2ndly. The anatropal ovule, where the micropyle lies near the hilum, and where the raphe or vascular bundle of the funiculus runs along one side of the ovule, as in Cucurbitaceæ, Irideæ, Liliaceæ, Impatiens, Viola, and Orchideæ. In this, as well as in the first-mentioned kind of ovule, the chalaza, that is, the place where the vascular bundle of the funiculus terminates, lies opposite to the micropyle, and the nucleus and embryo sac are not bent. The third species of ovule is the campylotropous ovule. In this case, the developement of all the parts has taken place only on one side. The micropyle lies near the hilum, the raphe is very short, and the embryo sac is bent. There are numberless intermediate forms and modifications of these types to which, in some cases, special names have been given; such names, however, are insufficient to characterise them. Careful drawings of different kinds of ovules will bring out their peculiar forms far better than the most ample written descriptions.

In considering the embryo sac, it is especially important to

attend to its relation to the nucleus. In the Orchideæ and Personatæ the nucleus is at an early period displaced by the embryo sac. In the Rhinanthaceæ, Orobanchcæ, Acanthaceæ, and Labiatæ, the embryo sac frequently produces sac-shaped elongations which absorb the parenchyma of the single integument, break through it, and often protrude into the hollow of the ovary. The only way of obtaining a clear view of this very interesting condition of the embryo sac is by means of a very thin longitudinal section carried through the middle of the ovule. Attention must also be paid to the presence of a real endosperm at the time of flowering. This may be observed in the Personatæ, Haloragcæ, and Hippurideæ. It is also important that the observer should satisfy himself as to the existence of peculiar cells at the apex or at both extremities of the embryo sac.

There are many other collateral organs of the flower, such as the imperfect stamens, the nectaries, the disc, &c., to which it is not necessary more particularly to refer. Whoever carefully follows the directions here given, cannot possibly overlook any such organ if it be present.

The directions given for the examination of the ovary will apply to the examination of the ripe fruit. Particular attention must here be paid, in an anatomical point of view, to the changes in the formation of the tissue, to the absorptions which are found to take place, &c. Morphologically the form and the nature of the dehiscence of the fruit will be of importance, and it will be necessary also to observe the changes of the other parts of the flower, and to ascertain whether they fall off soon after the period of flowering or whether they remain behind, and what effect they have on the form of the fruit or upon its condition.

The ripe seed is examined in the same way as the ovule, and in order to obtain a correct idea of its morphology, we have to consider its form and the nature of its outer surface. By means of thin transverse and longitudinal sections, the observer will be convinced of the transformation of the single or double integument into the testa of the seed, and will satisfy himself of the presence or absence of the pre-existing nucleus, the

tissue of which, when it exists in the fruit, is called the perisperm, or exterior albumen, as in the Nymphæaceæ. By means of these sections he also ascertains the presence or absence of the peculiar albumen or endosperm, a kind of parenchyma which is formed in the interior of the embryo sac; and lastly, he learns the nature of the cells of the embryo itself.

In making these investigations, the contents of the cells must be tested with iodine, and the iodized solution of chloride of zinc.

In examining the embryo itself and its position in the ripe seed, it is often advantageous to divide it into two equal parts, and to take a moderately thin transverse section of it. It is a good plan to soften hard seeds by soaking them in water for twenty-four hours. It will often be advantageous to detach the whole embryo, and to treat it separately from the seed. In difficult cases it must be examined on all sides with incident light under a low magnifying power, and must be illuminated in many different ways. The embryo of dicotyledonous plants will seldom be difficult to examine. The parts to be distinguished are the axis and the cotyledons. The axis is that part of the embryo which terminates in the direction of the micropyle, in the form of a little root, and the other end of which forms the plumule. The cotyledons proceed from this axis. Many Coniferæ have more than two cotyledons; the two cotyledons of the Lime are only leaves with many deep incisions; the Orobancheæ, Monotropa, and, amongst monocotyledons, the Orchideæ, have no cotyledons. The plumule is highly developed in some plants. In Tropæolum, for instance, two complete leaves are to be found. In other plants, on the contrary, such as Pedicularis, Impatiens, and Hippuris, it is only to be seen in the form of a slight protuberance between the cotyledons. The embryo of the Walnut has, in addition to the two cotyledons, numerous buds which appear in two longitudinal rows.* The radicle of all dicotyledons which I have examined is furnished with a root-cap. Pith and bark are, in all of them, separated by the cambium-ring; in many cases, as in the Oak, the Walnut,

* *See* Schacht's "Observations on the germination of the walnut," in his "Beiträge zur Anatomie und Physiologie der Gewächse," Abschn. VII.

and Viscum, some vessels are to be found even in the embryo. The embryo of monocotyledons presents greater difficulties—difficulties, in fact, which frequently can only be got over by attending to the developement of the embryo. Accurate longitudinal sections are here of great importance; they exhibit, in the Gramineæ, the developement of the lateral roots. The radicular end of the embryo of monocotyledons does not itself become a root. Monocotyledons, therefore, have no true tap-root. In the grasses, the sheath from which the young plant is protruded, must be looked upon as the first leaf of the embryo-plant. In some Palms, two or three leaves remain sheath-like. The formative tissue which lies under the plumule of the embryo of monocotyledons, I designate the *embryonic layer;* from it is developed, at a later period, the cambium-ring of the stem; from this tissue originate also the vascular bundles, and the lateral roots of the germinating plant also proceed out of it.

The form and position of the embryo, and the presence or absence of albumen are of importance in systematic botany.

The motion of the juices of the cell must not be passed over in silence. It is not to be seen in all plants, although it may be conjectured that it is present in all living vegetable cells. It may be seen in the simplest form in the hairs of the roots of Hydrocharis morsus-ranæ, for which purpose a perfectly fresh plant must be taken on a warm summer's day; those hairs on the roots which hang down in a flaccid manner will not exhibit the motion, but it can generally be found in those which stand out horizontally from a long thin root. A piece of one of such roots must be brought under the microscope and placed under a covering glass, and one particular hair must be watched continuously and attentively. It is seldom necessary to wait long; the motion is frequently interrupted just at first, but generally begins afresh in a few minutes. The stream flows along the walls of the cell, and is clearly seen to bend back again at the apex of the hair. Thin sections of the young leaf of the Hydrocharis, and of the leaves of Stratiotes aloides and Vallisneria spiralis, exhibit the same motion. In Vallisneria, large granules of Chlorophyll are carried along by the stream. The motion in the hairs of the stamens of Tradescantia is more

complicated. In these hairs different currents may be distinguished: the larger currents traversing the walls, and the smaller currents passing from the cytoblast to the walls; the direction of the latter frequently changes; they cease, and new ones form themselves. The hairs of the young ovaries of Œnothera and Clarkia exhibit similar movements. Warm, clear days and perfectly fresh plants are necessary for observing these movements. The movement of the contents in the parenchymatous cells, as in the Snowberry and in the youngest cells of the endosperm of Pedicularis, &c., is much less frequently seen; it depends upon a peculiar condition of the cells which may, by good fortune, be sometimes met with. I have observed, on two occasions only, but then in the greatest perfection, a very complicated movement in the prolongation of the embryo-sac of Pedicularis sylvatica. Any person who has carefully observed these appearances, if it be only twice in his life, will easily be convinced that they cannot be explained upon the supposition of any vascular system in the interior of the cells, but that the motion proceeds from a fluid, which is separated from, and does not mix with, the rest of the fluid contents of the cell. By applying iodine, or iodine and sulphuric acid, the fluid is turned yellow, and the motion then ceases, but there are many cases (Chara for instance) in which syrup does not stop the motion; the primordial utricle generally becomes somewhat contracted, and separates itself from the cell-wall, and the motion becomes slower.

On the method of investigating the developement of Plants.—In tracing the developement of a plant, it is necessary, if the inquiry is to be of any scientific value, to go back to the primary origin of the plant, or of the part under investigation. In tracing the developement of the embryo, therefore, it is necessary to show with certainty the origin of its first cell; the developement of the flower must be traced from the appearance of the floral axis, as a simple round cellular little body in the axil of the bract. In conducting the investigation, care must be taken not to overlook any matter of importance; where the investigation is complete, *i. e.*, where the developement is followed out in all its successive steps, great service is rendered to science;

and it is by so doing alone that clear ideas upon the subject can be formed. In questions relating to development fresh plants only must be used.

It would be almost impossible, from their variety, to point out the method of tracing the development in the individual groups of cryptogamous plants. I will only shortly mention what I consider, at present, to be most requisite for the advancement of science in this respect; and the first thing to be mentioned is *inquiry as to the germination of cryptogamous plants in general.* Hofmeister's work on the development of the higher Cryptogamia will serve as a model for this inquiry. In inquiries of this nature it would be necessary that attention should be particularly directed to the effect produced by the antherozoids upon the first cell at the bottom of the pistillidium, or at the bottom of the germ-organ, and wherever it is possible the nature of this effect should be followed out. No one, however, should undertake original investigations of this nature who has not had considerable experience, but at the same time it would be very instructive for persons of less experience to repeat the experiments of Hofmeister, Mettenius, and other well known observers. For the investigation of the germination of Ferns, which I have myself followed out, I can give a few hints. I may mention, as a point of great importance, the development of the reproductive organs, and the origin of the spores within the sporangia, the existence of gemmæ, their origin and development, and lastly, the development of the true antheridia, and of their phytozoary cells, must also be considered.

Besides the above-mentioned inquiries, many very interesting questions might be suggested, depending upon the peculiarity of the particular group or even genus. Late investigations have rendered the formation and germination of the spores of Algæ questions of great importance. There are many Algæ in which spores differing widely from one another are known to exist. These spores are primarily divided into zoosporés and motionless spores. The zoospores originate within a mother-cell, sometimes singly, sometimes in greater numbers; they are furnished with vibratory cilia, the number and position of which is different in different plants. The zoospores of Vaucheria are covered with cilia

over their whole surface; in Œdogonium the cilia are collected at one spot like a brush; the greater number of Algæ, however, have only two or three cilia situated near one another. The zoospores generally emerge all together from a fissure in the mother-cell; they continue in motion for a greater or less time, according to the species; the motion becomes gradually slower, the spore elongates, the cilia disappear, the spore lies still and germinates, and a new Alga is produced from it. It is often difficult to distinguish a zoospore from an Infusorium. It is necessary to have thoroughly observed the origin of the zoospore in the interior of the cell of an Alga, as well as the production of a new Alga by its germination, before the observer can be quite secure from mistakes; generally, however, the motion of a zoospore is different from that of an infusorium; the former is much more regular. In order to be able to observe one particular zoospore for any length of time, it is advisable in the first place to lay the threads of the Algæ which contain the ripe zoospores across one another, and to cover them with a covering glass, so that when the zoospores appear they are confined within a limited space. These observations can only be made in summer, and upon clear days; the zoospores generally escape from the mother-cell early in the morning. The red show, (Chlamido-coccus pluvialis), which may be kept dried in paper for years, germinates, even in winter, if brought into water, in the space of from twenty-four to forty-eight hours. A fragment of the green or red mass which is formed by these unicellular plants should be put into a watch-glass with water, and placed in a warm room exposed to the light. Here also the escape of the zoospores takes place in the morning, or at least in the early part of the day, more frequently than in the afternoon.

In addition to the peculiar zoospores above-mentioned, the germination of which is in most cases known, we find, under certain circumstances, in the same tribe of plants, smaller cells, called Microgonidia, which are endowed with motion, and are generally furnished with vibrating cilia; the germination of these Microgonidia has not yet been observed; they are probably analogous to the small cells of a similar nature which occur in the antheridia of the Fucaceæ and Florideæ, and to the

small cells of the Fungi and Lichens which have been called spermogonia. I have observed the zoospores in Ulothrix and Chlamidoeoeeus, as to which I would refer to my work "Die Pflanzenzelle," p. 121, Plate II., Figs. 20—39. The most important works upon the subject of zoospores are those of Alexander Braun ("Die Verjüngung in der Natur"), Thuret in the "Annales des Scienees Naturelles," Vol. XV., No. 4; F. Cohn. "Nova acta Academ," L. C., Vol. XXII., and N. Pringsheim ib., Vol. XXIII.

In Spirogyra the reproduction of the plant during the winter is effected by means of motionless spores which originate from the copulation of two cells; in summer the plant is reproduced by the separation of single cells (which become independent plants) and perhaps also by means of zoospores. The same relation between the zoospores and the motionless spores seems to obtain generally; by means of the former, which germinate immediately, the plant is reproduced in Summer; by the latter, which generally appear in the Autumn, the existence of the plant during the Winter is secured.

Amongst the Fungi and Lichens also attention should be directed to the different kinds of spores, and the mode of their formation and germination should be observed. This will afford a large field for investigation. In the higher Algæ, inquiry might be made into the nature of their growth, and especially as to the mode of thickening of their perennial stems. In the fungi, the effect of chemical agents upon the cellular membrane, in its old and young state, might be observed. In the leafy liverworts, an examination into the mode of developement of the perigone, as to which few accurate observations have been made, would be desirable; and with respect to all the Cryptogamia which have stems and leaves, an investigation of the developement of those organs would be very valuable.

In order to bring about germination in ferns, the best plan is to take a large fragment of a frond with ripe spores upon it, to place this fragment upon moist garden-mould in a flat earthenware vessel, and to cover it with a glass; the mould must be kept sufficiently moist, and the vessel placed in a tolerably warm shady place. After a time, varying from a fortnight to five

weeks, the spores generally begin to germinate; the first indication of which is a green parenchymatous expansion. Pteris serrulata germinates very easily. Some spores should then be taken up and rinsed with water. The antheridia in the younger specimens are very beautiful. When the germ has assumed a leaf-like form, transverse sections must be made with the section instrument: this is important when it is wished to observe the germ-organ and its developement; the germ-organ is closed at first, and afterwards opens. Accurate observations as to the origin of the primary cell within this germ-organ, and of the relation between the germ-organ and the spiral filaments or antherozoids would be of the highest importance to science. In examining the spiral filaments, it is important to observe their developement, their mode of escape from the antheridia, the number of their coils, the manner in which they are covered with cilia, the nature of their movements, and the manner in which they are affected by chemical agents.

The spores of liverworts generally germinate very easily in white moist sand under a glass. The genus Pellia germinates in a few days. Those spores which have a tough cuticle require a somewhat longer time. The spores of the Equisetaceæ only germinate when quite fresh; the examination of the pro-embryo is carried on in the same manner as in ferns. The developement of the pistillidia in mosses and liverworts may best be seen by taking a thin longitudinal section through the middle of the young stem. They are found, like the germ-organ of ferns, to be always closed in the first instance, and afterwards they open at their apex. In order to follow out the developement of the spores, thin longitudinal and transverse sections must be taken from time to time through the situs of the spores, from the earliest stage until the spores are ripe. The use of re-agents will here be essential. Blasia and Pellia are particularly well adapted for tracing the developement of the spores. In order to trace out the origin of gemmæ, it is necessary to observe the transformation of certain cells of the mother plant, and their subsequent developement into gemmæ, which must be done, either by means of longitudinal and transverse sections, or by carefully detaching the particular parts to be observed. In

Blasia the gemmæ remain for some time united to the mother-plant by a many-jointed cellular stalk. In the liverworts the pistillidia always make their appearance before the perigone or cup; the formation of the latter seems not to take place until the rudiments of the fruit have been formed in the interior of the pistillidium. The perigone is not formed of leaves grown together; it originates in the form of an annular swelling around the pistillidium, as may be seen in Liochlæna lanceolata and Frullania dilatata.

The developement of the stems and leaves of cryptogams, as well as of their vascular bundles, must be investigated in the same manner as in the case of phanerogamous plants.

On the investigation of the developement of the Stem, the Root and the Leaves, and of the Vascular Bundles contained in them.—In tracing the developement of the stem and leaves, two modes of proceeding may be adopted. The first is to examine the plant at the time of, and subsequently to, germination; the other is to follow out the developement of the bud, and of the young branch. In order to arrive at a satisfactory result, both methods should be pursued. In both it is necessary, in the first instance, to take very thin longitudinal sections directly through the middle of the apex of the stem. If a section is thus made, the apex, whether it originate from a germ or a bud, will be found to be a small closed protuberance of a more or less conical shape, clothed with a delicate epidermis, and underneath this protuberance will be found a tissue, consisting of small cells quite filled with a granular substance which is rich in nitrogenous matter. This tissue loses itself lower down in the different tissues of the stem, and is therefore in direct connexion with the cambium-ring. The vascular bundles originate in the cambium-ring, around which, and in which, and by means of which, these bundles become further developed; on this account the vascular bundles are always most fully developed in the *lower* part of a branch, where the growth always takes place from below upwards; this is very remarkable around the wood-ring at the time of the unfolding of the buds of Dicotyledons. If a very careful longitudinal section be made through the apex of a young branch, the age of the cells may be accu-

rately investigated; the deeper they lie the more fully developed they will be found to be, both in length and breadth, and in the degree of their thickening; they are younger and less developed in proportion as they are nearer to the apex. If a section of this nature is treated with iodine and sulphuric acid, the lower parts of it immediately become blue; towards the apex this change of colour takes place quite gradually, and passes through the most various shades of yellow, and through red and violet, to blue. The conical end of the stem frequently does not turn blue for many hours, but it becomes rose-red on the application of sugar and sulphuric acid.

Beneath this conical protuberance, which may be called the terminal bud, or Punctum vegetationis, and on both sides of it, if the section be well made, are to be seen other small protuberances, which are covered with the same delicate epidermis as the Punctum vegetationis, and which consist of cells of the same nature as those forming the tissue of the Punctum vegetationis. These small protuberances appear to be more developed in proportion as they are situated lower down upon the stem; they may easily be seen to be the rudiments of leaves. Shortly after the appearance of these rudiments of leaves, and in fact in their axils, a similar wart-like protuberance is often produced, which becomes an axillary bud. The leaf is generally developed without delay; its axillary bud, on the other hand, remains at rest for a time, and then becomes developed into a branch or a leaf. The apex of the leaf always dies first; it often becomes developed into a mucro. In dicotyledonous plants, whilst the mid-rib and veins continue to be developed, the edge of the leaf ceases to form new cells; the edge thus becomes dentate, serrate, &c. The principal veins proceed from the mid-rib; the secondary and inferior veins, which do not extend to the edge of the leaf, then make their appearance in succession. When the foundation of all the important parts of the surface of the leaf has been laid, it begins to grow, apparently with tolerable regularity, by cell-extension. For tracing the development of leaves, young buds should be chosen, and the young leaves removed one by one until the Punctum vegetationis is reached, and the latter must then be brought under

the simple microscope. By this means the steps of developement are followed out successively; the youngest leaves naturally lie close under the Punctum vegetationis. Longitudinal sections through the middle of the bud are also necessary in tracing leaf-developement; care must be taken that such a section pass accurately through the middle of the cone of the Punctum vegetationis, since oblique sections give rise to erroneous ideas; transverse sections at different heights are also necessary in order to become acquainted with the position of the leaves in the bud. The mode of growth must also be particularly observed; it will always be found that the apex of a leaf is the part at which the formation of new cells first ceases. The origin of axillary buds must also be observed; it would seem that they are always formed shortly after the rudiments of the leaf itself. This is the case in deciduous trees, in acerose-leaved trees, and in the Orchideæ. Lastly, attention should be directed to the formation of the veins in succession one after another, and to the mode of growth of the peduncle. A comparison of the manner of developement of different forms of leaves will afford interesting results.

The *Axillary bud* consists at first only of a small conical protuberance, which afterwards becomes elongated, and forms what may be called the bud-stem; the rudiments of leaves originate under its apex; and in those cases where the bud does not immediately become developed into a branch or a leaf, these rudiments ultimately form scales. Under the protection of these scales the Punctum vegetationis rests for a period, after which new leaves are formed beneath it, which either continue covered by the scales during the winter, or break out immediately, and complete their developement, as in the case of what are called the second shoots of trees. In the leaf-buds of annuals which are immediately developed scales are seldom found. Flower-buds cannot at first be distinguished from leaf-buds.

Besides the terminal bud and axillary buds, there are also adventitious buds, which originate in the cambium-ring of the stem and of the root, or are formed in the leaf-parenchyma, as in Bryophyllum, Cardamine pratensis, Malaxis paludosa, and in many ferns. These adventitious buds consist at first, like all

other buds, of a small cellular protuberance. The developement of them may be examined by means of longitudinal and transverse sections; and careful attention must be paid to the connexion between the rudiments of these buds and the vascular bundles of the part from which they originate. The Punctum vegetationis of a stem-bud sometimes becomes divided into two or more parts, each of which may be developed into a branch or a flower. The stem of Selaginella ramifies in this manner as well as the Rhizome of Epipogium and Corallorhiza. In the Beech, the two flowers which are enclosed in one capsule also originate in this manner. The division of the Punctum vegetationis of stem-buds and root-buds may probably also be observed in many other cases. Good longitudinal sections made at different periods of developement are here very necessary, and the sections must pass precisely through the middle.

In order to follow out fully the developement of buds a sufficient time must be devoted to the inquiry; by this means the observer ascertains how long a time is required after the rudiments of a bud are formed before it becomes developed into a branch or flower, he also becomes acquainted with the different periods of existence of different buds. For instance, the bud which forms the cone of Abies pectinata requires two whole years to perfect itself. It begins to be formed late in the summer, and almost contemporaneously with the leaf in the axil of which it originates. In the succeeding spring this bud forms its scales; in the summer the rudiments of the cone are formed under the protection of the scales, and continue under their cover during the winter; in the next succeeding spring (*i.e.*, the second spring), it bursts forth, and in the autumn the cone ripens.

There are many plants in which the buds which originate near one another in the axil of the same leaf become developed in a different manner, and at different periods; this is the case with the Lime and the Vine.

With regard to the morphology of buds, it is necessary to observe the position and arrangement of the leaves, and to notice also the scales, and the manner in which the latter pass by degrees into true leaves. With regard to the anatomy of

buds, it is necessary to follow out accurately the developement of the stem. In order to render the investigation valuable, every important individual part of the bud, and the changes which take place therein, must be accurately and progressively examined.

Germination must be followed out in the same manner as bud-developement.

The formation of the stem and the origin of the root must be observed. In dicotyledonous plants the radicle itself becomes the first root, and forms the proper tap-root. In Monocotyledons, on the other hand, one or more adventitious roots originate in the tissue of the radicle.

If a thin transverse section is now taken close under the Punctum vegetationis of a young branch, there will be found, in dicotyledonous plants, a number of dispersed vascular bundles, the ligneous cells of which are turned towards the pith, and the cambium towards the bark; these vascular bundles are separated by a mass of parenchyma, often of great width, which unites the pith and the parenchyma of the bark. In a very young state of the plant, the wood-cells and vessels are scarcely distinguishable from the cambium, and the liber-cells are generally not yet present. At a subsequent period the different parts are more clearly defined; the liber-cells appear on the outside of the cambium, the vessels become extended, and the parenchyma, which at first separated the vascular bundles from one another, becomes reduced to a narrow remnant constituting the primary medullary rays. A closed ring of wood is now formed, increasing in circumference yearly by additions from the cambium-ring, which on its inner side forms new wood, and on its outer side new bark. In cryptogamous and monocotyledonous plants, where the cambium of the vascular bundles does not coincide with the cambium-ring, the stem increases in thickness, but its vascular bundles do not become thickened, but ramify in the cambium-ring; the number of them, therefore, which is met with in a transverse section increases with the age and with the thickness of the stem, as may be seen in many Palms. When the activity of the cambium-ring ceases, the thickening of the stem, or of the root, also terminates. In order to trace

the formation of the young wood, it is necessary, both in spring and in summer, to make transverse and longitudinal sections in two different directions; the sections must be extremely thin, and the cambium especially must be cut through quite smoothly. It is sometimes advisable to place these sections for a few minutes in a weak alkaline ley. By so doing, the cambium cells frequently become more transparent; glycerine also might be advantageously employed. In the young wood-cells of Picea vulgaris a spiral band may be clearly seen, as well as the gradual formation of the pitted vessels.

Very thin longitudinal sections taken from the apex of a young twig, such as those which have been recommended for the examination of the stem and leaves, afford also sufficient information as to the origin of the vascular bundles. It will be seen how all the parts, the cambium, the wood, the vessels, and the liber-cells, originate underneath the Punctum vegetationis; and in going downwards from this point it is possible, by means of very careful sections, to follow out the developement of these different species of cells, and to perceive, especially in the vessels, the gradual formation of their peculiar thickening layers. It will be seen further, how the increase in number of the cells takes place principally in the tissue underneath the apex of the stem, and in the cambium of dicotyledonous plants; and how, on the other hand, the growth of the parts further removed from the Punctum vegetationis depends chiefly upon an increase of size and elongation of the cells. *Cell-multiplication* and *cell-extension* are essentially different. This distinction must always be borne in mind in questions relating to developement.

At the places where young leaves originate the passage of the vascular bundles out of the stem into the leaf may be seen, in dicotyledonous plants. These prolongations of the vascular bundles, out of which the veins of the leaves are formed, originate almost contemporaneously with the first rudiments of the veins. I have seen the same thing in Epipogium and Goodyera, which are monocotyledonous plants.

The vascular bundles of all plants, when present, generally appear to have a certain connexion with one another, which connexion depends upon the manner in which they originate.

It is, therefore, incorreet to suppose that the stem is formed out of leaves grown together; the nature of its developement shows direetly the eontrary. In the dicotyledonous embryo the simple central portion—that is, the axis—appears first; the two cotyledons proeeed from it on either side, and between the cotyledons lies the plumule, whieh answers to the Punctum vegetationis of the apex of the stem. The first two leaves, therefore, are formed to a certain extent out of the stem by the division of it, and not the stem by the growing together of two leaves. The further progress of developement corroborates this. At the points where new leaves originate there is formed contemporaneously with them a side branch of a vaseular bundle of the stem, the growth of which progresses equally with, and in the same manner as, the growth of the leaf. The leaf, therefore, reeeives its vascular bundles from the stem, but a new vascular bundle never originates in the leaf to unite with the vascular bundles of the stem. The same rule holds with respect to the origin of new buds in the axils of the leaves; and with respect to the cambium-ring of the stem and of the root. The first rudiments of new buds, as well as of side-roots, always originate out of the vascular bundle of the stem. The only places where the formation of much new substance for the buds and leaves takes place, are, the apex of the axis, and the eambium of the vascular bundles of the stem.

I have dwelt longer upon this part of the subjeet, beeause the point, which is one of much importance, has by no means received the attention it deserves, and but few well-grounded observations have been made upon the subject. It is of the greatest importance that very accurate sections alone should be used; oblique sections have been the cause of much error. The best way of guarding against this error is, to make the finest possible longitudinal sections through the apex of the stem, to place them together under the microscope, and to select those sections which appear to be cut quite perpendicularly through the stem, as well as exactly through its middle. When such a section is properly made, the Punctum vegetationis always appears in the form of a small cone; if this is not the case the section is either not cut perpendicularly through the stem, or

not exactly through its middle. It is, moreover, indispensable to distinguish accurately between the increase in number of cells and their increase in size.

For tracing the developement of roots, the rules given for the stem will generally apply. The root also grows at its apex, but it is covered with a root-cap, *i. e.*, with a layer of dead cells; it cannot, therefore, form leaves in the same manner as the stem, the conical termination of which is uncovered. In the root, therefore, it is necessary to observe the origin of the root-cap and its subsequent developement. The developement of the tap root may be traced in the embryo of dicotyledons. The origin of adventitious roots is to be observed in the embryo of Monocotyledons, and also in the stem and in the roots of plants. Roots generally ramify by means of adventitious roots; the bifurcation of the conical termination of the root, which is to be seen in the tuber of the Orchis, in the aerial roots of the Cycadeæ, and in the swollen knobs of the Alder, is not of frequent occurrence. The mode of formation of the root-bud in the cambium-ring of a stem, or of a root, corresponds exactly in its earlier stages with the mode in which adventitious stem-buds originate; both break through the bark; the appearance of the root-cap, however, soon distinguishes the root-bud from the stem-bud; the latter does not begin to form the rudiments of leaves until it has broken through the bark.

On the method of examining the developement of Flowers.—In examining the developement of the flowers much greater difficulties will be met with than in tracing the developement of the stem, the root, and the leaves. From the minuteness of the object it is impossible always to regulate accurately the direction in which the sections are made, and on this account it is often necessary to select out of many sections those which happen to have been well prepared, and some experience is necessary to be able to distinguish the good sections from the bad. Still greater difficulties arise in the case of irregular flowers. The growth of the different whorls of leaves does not always progress simultaneously; the petals, although they are always formed before the stamens, frequently lag behind the stamens in their subsequent developement, and on that account are some-

times liable to be overlooked, and other difficulties frequently occur. Every beginner, therefore, should be advised, before commencing the study of the developement of irregular flowers, to make himself fully acquainted with the developement of regular flowers. The flowers of Œnothera, Clarkia, and Epilobium are well adapted for this purpose. In order to render the investigation more easy, it is necessary to select plants which have a spiked or clustered inflorescence, and moreover plants which have but few hairs. The longitudinal section through the middle of such a spike exhibits, simultaneously in the axils of the bracts, different stages of the developement of the flower. Moreover, when the flowers have but few hairs, the examination of them is much less liable to error, since air is often collected between the hairs, and this must first be removed with alcohol, the use of which, in such young specimens, is often not advisable. In examining the developement of the flowers, two modes of proceeding may be adopted. First, the parts of the flower in their successive stages may be prepared separately by the aid of the simple microscope; and, secondly, very delicate longitudinal and transverse sections, in certain definite directions, may be made through the whole of the flower. The second mode of proceeding is decidedly preferable; it is more rapid and more certain in its results; it affords a far more accurate insight into the internal condition of the flower and its parts; and lastly, after a little practice, it is far more convenient and easily managed. In preparing the separate parts, there is no security, notwithstanding the greatest dexterity in the use of the needle, against their being injured; and finally, the observations are rendered difficult by the fact that the parts of the flower must be viewed as *bodies*, by varying the adjustment of the microscope, and cannot, as in the case of the sections, be examined as *surfaces*. In many cases, as for instance in examining the developement of the flowers of grasses, both methods should be used.

In selecting specimens for examination, the youngest flowering branches should be taken. The longitudinal sections should be made with the unassisted hand. The section must be very thin, and must exhibit accurately the middle lamella of the flowering branch; and in examining it, the terminal bud and

the bracts beneath it must be observed. The first rudiments of the flower will be seen within the bracts in the form of a round cellular little body, precisely similar to the first rudiments of a leaf-bud; this cellular little body is the proper axis of the flower. The sepals will be seen in the form of little round warts, situated in the axils of the bracts, and in a circle round the cellular little body just mentioned. In cases where the section bisects the floral rudiments the apex of the floral axis is seen between the rudiments of the calyx in the form of a round or conical protuberance. In such a section the rudiments of the different whorls will be seen in succession one under the other.

Having gained some information by means of longitudinal sections, the observer must then prepare thin transverse sections, proceeding from the apex of the flower. It is often advisable to make these transverse sections in a direction somewhat oblique to the principal axis, inasmuch as the position of the floral rudiments with respect to that axis (*i. e.*, the common flowering stem) is generally somewhat inclined. This rule is particularly applicable to those flowers which are situated low down on the stem. It is important, for the purposes of investigation, that the transverse sections should be cleanly made, and that they should be exactly at right angles with the longitudinal axis of the floral rudiments. It is necessary, therefore, out of the number of floral rudiments through which one such section generally passes, to select those which appear to have been divided in the right direction. It is often necessary to make many sections before it is possible to obtain perfect specimens of the different stages of development. It is particularly necessary to follow out the development continuously step by step, and therefore it is a good plan to make an accurate sketch of the outlines of all successful sections, both longitudinal and transverse. If such longitudinal and transverse sections, taken from different plants in similar stages of development, are compared with one another, they cannot fail to lead to a right understanding of the subject. The simple microscope will be found to be indispensable for improving longitudinal sections by the removal of superfluous parts, as well as for detaching, for preservation, particular portions of transverse sections of the entire floral

rudiments. Repeated use of the razor is often necessary for rendering longitudinal sections more complete.

In examining the developement of a flower by the aid of a transverse section, the following points must be particularly attended to:—

1. The succession of the floral whorls, and their number.

2. The position of the parts of one whorl with respect to those of the preceding one. If these parts do not alternate with one another, the rudiments of the missing, perhaps suppressed, whorl must be sought after; if they are not found, it does not necessarily follow that there should be a malformation of the whorl.

3. The number of the parts of each whorl, and the manner in which they harmonize with one another. When one whorl consists of fewer parts than the preceding, a malformation of some organ may generally be ascertained by observing the position of the parts of the whorls *inter se;* in this case also the rudiments of the missing organs must be sought after; and they will occasionally be found in their right place in the form of inconspicuous excrescences. When, on the other hand, it happens (which, however, is seldom the case) that a whorl has more parts than the preceding one, the first thing to be inquired into is, whether the preceding whorl is complete, or whether the additional parts in question really belong to the whorl in which they are found.

4. The growing together, or adhesion, of the originally distinct parts of one or other of the whorls. This can only be observed by comparing good transverse sections taken at different periods of the developement, from which it will be seen that a real *growing-together* seldom occurs, but that the separation of the parts of a whorl frequently ceases at a certain point; in this way the gamopetalous corolla and the calyx of corresponding form originate, as well as the tube of the Anther in Ruscus, &c., and the ovaries of many plants.

5. The construction of the anthers—as to whether, up to a certain period, they are bilocular or multi-locular.

6. The parts forming the ovary. The superior ovary may originate in the form of a closed tube, but it may also be formed

out of one or more originally separate leaves. The number of these parts seldom stands in any definite relation to the parts of the preceding whorl. In very many cases it will be a matter of doubt whether the ovary is formed from the stem or from leaves. The inferior ovary must always proceed from the stem, inasmuch as it bears the whorls above it.

7. The origin of the placentæ and ovules, and the nucleus, coatings, and embryo-sacs of the latter. This investigation is of great importance; it shows whether the dissepiments of the ovary are true or false. In Œnothera, Clarkia, Epilobium, in the Cucurbitaceæ, in Pyrola, Monotropa, &c., they are false; and are in fact, formed out of the parietal placentæ. True dissepiments, on the other hand, originate from the folding inwards and growing together of the edges of at least two carpellary leaves, as is the case in Papaveraceæ and Nymphœaceæ. False dissepiments are much the most common.

Transverse sections of the stigma and style will seldom afford satisfactory information.

In longitudinal sections the following points must be attended to:—

1. The primary insertion of the parts of one or more of the whorls, and whether their position, at a later period of developement, has remained unaltered; or whether the parts of one or other of the whorls are pushed upwards. The formation of a disc, the origin of appendicular organs, the developement of hairs, &c., must also be attended to. The cupule of the oak and the beech is developed from a disk, which, during the period of its formation, produces leaves under its edge; these leaves, in the oak, are of a scale-like nature.

2. The developement of the ovary. The apex of the original flower-bud may extend itself into the hollow of the ovary, and form a free axile placenta, as in the Primulaceæ, Lentibulariaceæ, &c.; or the apex may extend itself in like manner, but become united with the pre-existing parietal placentæ, and thus make the lower part of the ovary multi-locular, whilst the upper part is uni-locular, and furnished with as many parietal placentæ as there are loculi in the lower part. It may happen also that the columella may become united with the introverted edges of

the true carpellary leaves, as in Papaver and the Nymphœaeeæ, or lastly, the columella may not be developed at all, in which case the ovary will be uni-locular through its entire length, as is the case in the Violet and the Cucurbitaceæ. These different modes of development of the ovary must all be attended to, as well as the point whether the ovary grows at its apex or at its base. The mode of formation of the style and stigma must also be observed.

3. The connexion of the canal of the style with the hollow of the ovary. This connexion can often only be clearly understood by tracing the development of the flowers; a comparison of good longitudinal sections in different stages of growth will leave no doubt as to the existence of the connexion. The development of the ovule is treated of in connexion with the origin of the embryo.

The use of the words "adhesion," or "growing together," in speaking of the union of the parts of flowers, as, for instance, in the case of the petals of gamopetalous flowers, frequently gives rise to erroneous ideas. The petals or sepals, which at first appear as separate parts, do not subsequently *grow together* at the bottom, but in the course of the development which takes place at their base, the *separation* subsequently ceases; it would, therefore, be more correct to speak of petals as *not separated* than as *grown together*. A true example of growing together does, however, take place in the stigmas of the Apocyneæ and Asclepiadeæ, in which cases the two stigmas of each ovary, which are at first completely separated, grow together and form one united stigma.

On the method of tracing the development of the Embryo.— In order to be at all successful in carrying on this most difficult of all anatomico-physiological investigations, it is necessary, in the first place, to be thoroughly acquainted with the construction of the ovary, the style, and the stigma of the plants to be examined, as well as with the development of their ovules; it is necessary, at least in some plants, to examine attentively the canal of the style of a flower which has not shed its pollen, as well as the canal of the style of a flower to which the pollen has been applied by the observer himself, in order to become

acquainted with the course of the pollen-tube, and the changes to which it gives rise in the canal of the style. Moreover, the condition of the ovule and of the embryo-sac at the period of flowering, before any pollen-tube has reached the ovule, must, in all cases, be fully examined, and the contents of the embryo-sac must be most carefully attended to, since it is only by these means that it is possible to form a correct judgement as to the changes subsequently produced by the agency of the pollen-tube.

In order to trace the course of the pollen-tubes from the stigma into the hollow of the ovary, the best plan is for the observer himself to apply the pollen to the flowers. One or more of the flowers should then be examined daily, by taking thin longitudinal sections from the middle of the style and ovary. By this means we ascertain the time which the pollen takes to protrude the tubes and press them into the ovary. Besides making good longitudinal sections, it is often advantageous to separate the walls of the canal of the style from one another by the aid of the simple microscope; in which case a large bundle of pollen-tubes may often be seen intermixed with the cells of the conducting cellular tissue, and this bundle of tubes may not unfrequently be traced into the hollow of the ovary by the help of a needle under the simple miscroscope. In plants with long thin styles, which soon wither, the attempt to follow the course of the pollen-tube without interruption will seldom be successful, but in plants with short fleshy styles it is frequently not difficult. The Orchideæ are the most favourable plants for this purpose. If the style of the flower of an Epipactis, to which the pollen has been applied about eight days previously, be examined in the manner above mentioned, the observer will be surprised at the extraordinary number of pollen-tubes, and he will easily be able to trace them in large strings, even as far as the ovules. Viola tricolor and Ribes nigrum and rubrum, are also good plants for the purpose; in the case of the former plant, withered flowers may be taken, and branched pollen-tubes will not unfrequently be met with. These branched pollen-tubes are found, even more frequently, in Fagus sylvatica and Œnothera muricata. No definite

mode of proceeding can be given for tracing the developement of the ovule, which must be regulated by the number and arrangement of the ovules in the ovary. Sometimes the transverse section, sometimes the longitudinal, will be most serviceable. The first appearance of the nucleus of the ovule out of the tissue of the placenta in the form of a conical cellular little excrescence, the origin of the coatings of the ovule in the shape of circular enveloping folds around the nucleus, the slight contemporaneous bending of the ovule, and the appearance and nature of the embryo-sac within the ovule, must all be noticed. In Hippuris and Myriophyllum, the ovules are without integuments, and are anatropal. They are also provided with a vascular bundle in the naked nucleus. In Thesium, the nucleus is also naked, but has no vascular bundles. As specimens of plants, the ovules of which are provided with integuments, may be mentioned Juglans, Taxus (in which the ovule is orthotropal), Impatiens, and the Rhinanthaceæ. In the latter, the ovule is anatropal, and the embryo-sac is extended into sac-shaped prolongations, which lie in the parenchyma of the integument. In Hydrocharis and Polygonum, Viola, Œnothera, and the Orchideæ, the ovules have two integuments. In Polygonum, the ovule is orthotropal, and in the Orchideæ, anatropal.

The ovules of many plants at the period of flowering are so large that they may be detached, placed upon the finger, and sections made of them; the direction of the cut must be particularly attended to. One side of the ovule must first be removed with an excessively sharp hollow-sided razor; the ovule must then be carefully turned round with a fine camel's-hair brush, and the other side removed in like manner by passing the razor steadily and slowly through the ovule, so that of the whole ovule there will remain only the middle lamella. The object must not be permitted to become dry during the process of cutting, and consequently the finger must be kept moist. The lamella, thus prepared, must be immediately placed under the microscope without a covering-glass. It will frequently happen that the section may be improved by a third or a fourth cut, made in the same manner; and although, in doing this, the object is sometimes spoiled,

nevertheless superfluous parts may often be successfully removed in this manner. The needle and the simple microscope will also often be of service. When it is possible, it is very desirable completely to detach the embryo-sac of a flower which has not yet shed its pollen The embryo-sac then appears in the form of a simple cell; but in most cases it is so tender that it is destroyed by the operation, or at least the cells which take their origin in it are spoiled. In this case, it is better to be satisfied with the thinnest possible longitudinal sections, and to study accurately the contents of the embryo-sac, remarking especially the presence or absence of cells therein, and their situation, if any. A solution of iodine is here desirable. The observer must not be satisfied with the preparation of one specimen, be it ever so successful; many sections, and these as perfect as possible, must be taken and compared with one another. It will then soon be seen whether or not a cellular formation is always to be found within the embryo-sac, even before the pollen-tube has penetrated the ovule, and it will also be seen what explanation is to be given with respect to these cells. In Lathræa Pedicularis and Hippuris, the mother-cells of the albumen are formed before the period of impregnation.

When the ovule and the condition of the embryo-sac before the shedding of the pollen has been observed, the same mode of proceeding must be adopted with the ovules of the flowers which have shed their pollen. In the Orchideæ, the ovules of which are very small and delicate, it is not possible to make sections through the ovules. They therefore cannot be used for investigating the origin of the embryo itself, that is, the examination of them can afford no positive evidence, either in favour of, or against, any one of the three different opinions entertained upon the subject.* On the other hand, the entrance of the pollen-

* According to the opinions of Schleiden, Gelcznoff, de Bary, and myself, the first cell of the embryo originates in the interior of the pollen-tube. According to Amici, Hofmeister, Unger, and Von Mohl, a cell exists in the embryo-sac before the shedding of the pollen, which cell is impregnated by the pollen-tube: that is to say, it is rendered capable of becoming developed into the first cell of the embryo. According to Tulasne, this first cell of the embryo is produced by the union of the pollen-

tube into the micropyle can be easily observed in these ovules. It is only necessary to remove the ovules of the swollen ovary of an orchis with a needle, which, after impregnation, may easily be detached from the placentæ, and there will often be discovered from one to five pollen-tubes in one micropyle. It will often be necessary to drive out the air by a gentle pressure with the compressorium. Euphrasia officinalis may well be employed for the same purpose. It is only necessary to tear open with a needle the ovary of a flower which has just withered, and almost every ovule will be found to contain a pollen-tube. The same thing may be seen in Veronica serpyllifolia, by making thin transverse and longitudinal sections through the ovary of a flower which has shed its pollen. In some plants, the entry of the pollen-tube into the ovule is for many reasons more difficult to observe, partly because that part of the tube which hangs out of the ovule is very quickly absorbed, as is the case in Ornithogalum and Hippuris, and partly because, from the position of the ovule itself, they are cut away in making the section, as is the case in the Œnothera. In these cases, when the specimen is well prepared, the pollen-tube is found either inside the micropyle, or, as in Œnothera, at its passage through the nucleus.

Whenever it is possible, the embryo-sac and the pollen-tube should be completely detached, for I am persuaded that this is the only way to arrive at a complete and final solution of the very difficult question under consideration. It is not always possible completely to separate the apex of the embryo-sac together with the pollen-tube which has penetrated it, from the surrounding tissue. It may often be effected in Canna. In this plant I have often drawn out the pollen-tube uninjured from the embryo-sac, and found the end of the tube already swollen; in Taxus I have done the same with pollen-tubes which had already developed cells in the corpusculum.* The Personatæ

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tube with the membrane of the embryo-sac. The proofs adduced by Hofmeister, in support of his view, are all of a negative character. In my treatise "Die Pflanzenzelle," I have discussed the matter fully.

* De Bary has also pointed out the direct passage of the pollen-tube into the embryonic vesicle (*i.e.*, into the first cell of the embryo) in Canna and in Pedicularis.

are in every case the most favourable plants with which to institute this most important investigation, especially those plants in which the apex of the embryo-sac does not even in a late stage become filled with cells, and where the nature of the ovule permits of an entire separation of the apex of the embryo-sac. Having made many trials, I can recommend for this purpose Lathræa squamaria, Pedicularis palustris, and particularly Pedicularis sylvatica. I have always been very successful with Lathræa and the last-named plants. The peculiar construction of the ovule itself, with which the observer must make himself fully acquainted, is, in the plants above mentioned, particularly favourable for the investigation now under discussion, and the form of the ovule will afford a guide as to the direction in which the section must be made. The middle lamella of the ovule, when obtained in the manner pointed out above, must first be placed under the compound microscope, and examined on both sides with a power of not less than 200 diameters. The lowest eye-piece should alone be used in this examination.

When it is thought that the section may be improved, the side of the section and the particular point to be operated upon must be noted, and the lamella cut accordingly. The specimen must then be again examined, and when the section is considered satisfactory, it should be brought under the simple microscope, with a magnifying power of from 15 to 30 diameters, and the parenchyma surrounding the apex of the embryo-sac removed with the needle. Although the observer may carefully follow the above directions, it will seldom, if ever, be possible, at least with Lathræa, to separate completely and without injury the whole of the embryo-sac and its prolongations from the surrounding tissue. In tracing the developement of the embryo, it is sufficient if the apex of the embryo-sac is completely separated, so that the observer may be able fully to study the relation of this apex to the pollen-tube which has penetrated it; and with a little perseverance and dexterity this separation of the apex may generally be successfully effected. In Pedicularis and Lathræa there will seldom be found to be any considerable portion of the pollen-tube on the outside of the embryo-sac; it

very soon becomes softened and dissolved in the micropyle. In Lathræa two pollen-tubes will often be met with in the cell-less apex of the embryo-sac; of these one only reaches the endosperm in order to become developed into the embryo.* These pollen-tubes are generally closed above, and cannot always be recognised as the remains of the portions of the pollen-tubes which are found in the micropyle. On the other hand, by accurate adjustment, correct illumination, and careful examination of the specimen on both sides, it may always be seen that the sac which lines the cell-less apex of the embryo-sac, and which, reaching to the endosperm, becomes the embryo, can never originate inside the embryo-sac, but must have penetrated into it from without, because the upper end of it, which is generally round and closed, always projects, often very prominently, above the apex of the embryo-sac; and, moreover, at the place where the pollen-tube has penetrated, there may be observed, in most cases, a decided introversion of the membrane of the embryo-sac, caused by the penetration of the pollen-tube.† By preparing a great number of specimens in the manner above mentioned, I traced the origin of the embryo in Lathræa and Pedicularis from the formation of the first cell in the interior of the pollen-tube to the appearance of the two cotyledons. When the young embryo has developed a few cells, the endosperm should be removed; the observer will then be easily and certainly convinced that the first cells of the embryo originate in

* There are other plants besides Lathræa in which two, or even more, pollen-tubes frequently penetrate into the micropyle of one ovule, and this fact affords an explanation of the manner in which Amici and Hofmeister have here and there been led into error; for when the apex of one pollen-tube lies sideways on the embryo-sac, the rudiments of the embryo being already present in the interior of the embryo-sac, it is necessary to search for a second pollen-tube, which will be found if the investigation be properly conducted.

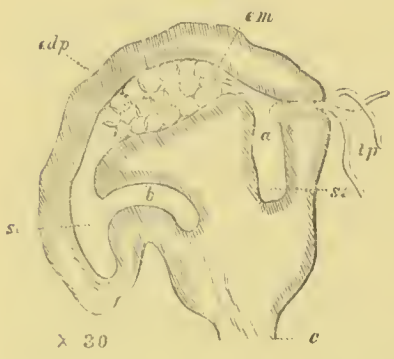
† The nature of the detached apex of the embryo-sac deserves a more accurate examination than it has hitherto received. Tulasne has very accurately described the introversion of the apex of the embryo-sac by the pollen-tube in many of the Personatæ. His opinion, therefore, does not coincide with the theory of Amici and Hofmeister. De Bary has fully confirmed my observations on Pedicularis sylvatica.

the interior of the sac above mentioned; and if he has previously satisfied himself of the identity of this sac with the pollen-tube, no further question can be raised as to the origin of the embryo, that is to say, as to the origin of its first cells in the interior of the pollen-tube.*

The importance of the question, and the great difficulty of its solution, have induced me to enter into the above details; it was particularly necessary to lay down a complete and definite mode of proceeding, since the methods hitherto attempted by other observers are, in my opinion, insufficient. I place no weight, for example, upon the examination of the Orchideæ; the cells of the integuments in these plants are too apt to mislead. The case is not much better with any other plants which do not admit of the separation of the apex of the embryo-sac; such plants I should never recommend to be employed for this investigation, because I consider the complete removal of the apex of the embryo-sac to be the first and most indispensable requisite for a solution of the above question. I think that this is the only way in which it is possible to obtain correct ideas of the true relation of the pollen-tube to the embryo-sac.

Figs. 60, 61, and 62 are explanatory of the matter just treated of. Fig. 60 represents a very perfect longitudinal section through the ovule of Lathræa squamaria at the time of flowering. The nucleus of the ovule has been long since absorbed by the embryo-sac; the embryo-sac has peculiar prolongations (*a*) and (*b*) at either end, which sink deep into the single integument, nay, afterwards even break through it, and project into

Fig. 60.

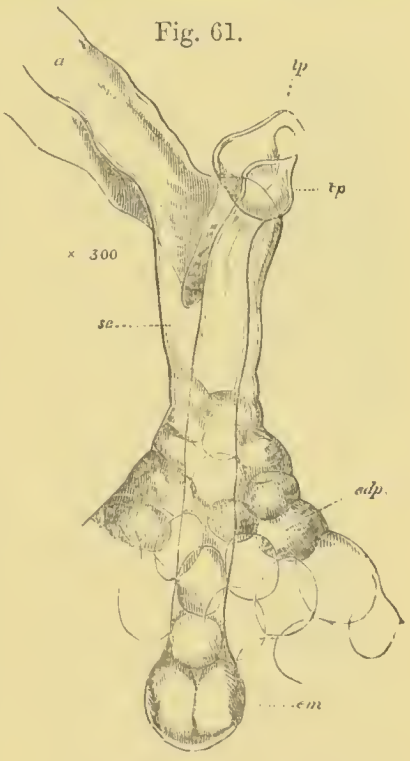


* It may be well to caution the reader that Dr. Schacht's views as to the origin of the embryo are by no means generally received. Unger's views upon the subject are given in the Appendix to this translation, and the reader may also refer to Hofmeister's work, "Die Entstehung des Embryo der Phanerogamen," and to Mohl on the "Vegetable Cell," translated by Henfrey.—Tr.

the hollow of the ovary. The middle part of the embryo-sac becomes filled with endosperm at an early period; the other parts of it never contain cells. The pollen-tube passes through the cell-less apex, which is connected with the front prolongation (*a*), and when it reaches the endosperm, swells and forms the embryo; in this flower many pollen-tubes are often found in the micropyle; (*c*) is the hilum of the ovule.

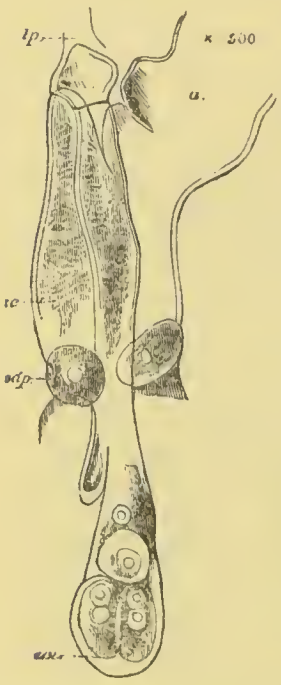
Fig. 61 represents the apex of the embryo-sac of Lathræa squamaria prepared in a perfect and uninjured state, drawn from a perfectly fresh specimen: (*a*) is a part of the front prolongation (*see* fig. 60). Two pollen-tubes have penetrated the embryo-sac, one has already swollen into a rudimentary embryo within the endosperm, the other has penetrated but not developed itself. Both pollen-tubes are seen protruding far out of the cell-less apex of the embryo-sac. The outline of this apex on the upper side is seen distinctly. On the under side it appears as a less defined line, which stands out clearly when the adjustment of the microscope is altered. Both pollen-tubes are somewhat swollen in the parts which project from the embryo-sac; they pass off by degrees into a thin ragged end. The part of the pollen-tube which lies in the micropyle is generally so much softened that it is not possible to detach a large piece of it; the pollen-tube which has penetrated the embryo-sac is frequently broken off at the end which projects from the embryo-sac. Two pollen tubes are often found in the embryo-sac of Lathræa, but one only develops an embryo. Fig. 62 represents a preparation quite as perfect as that shown in fig. 61, also drawn from a perfectly fresh specimen: (*a*) is

Fig. 61.



the front prolongation of the embryo-sac, into which only one pollen-tube has entered, which projects for some distance out of the embryo-sac, and is broken off. The outline of both sides of the embryo-sac is seen. From the appearance of the outline of the under side, it would appear that the membrane of the embryo-sac was pushed inwards by the entrance of the pollen-tube. The pollen-tube, after having reached the endosperm (which is not represented in this fig.), has formed many cells, which are the foundation of the embryo. The cytoblast has already become divided in the two lower cells. Over these young cells the pollen-tube itself forms a small recess. Specimens such as those represented in figs. 61 and 62 may be kept in chloride of calcium for a length of time without becoming deteriorated in any way, so far as relates to the pollen-tube and the embryo-sac.

Fig. 62.



Observations on the embryo will be more certain and valuable in proportion as the separate steps of the developement of the embryo are more fully and completely followed out. I recommend the observer not to hurry himself, since one perfect observation is far more valuable than ten imperfect ones. In dicotyledonous plants, the first appearance of the two cotyledons will be seen in the form of small excrescences upon the originally round rudiments of the embryo. The formation of these cotyledons, and of the plumule between them, must then be observed, as well as the origin of the cambium-ring in the axis of the embryo, and the formation of the root-cap around the end of the radicle; particular attention must also be paid to the condition of the embryo in the ripe seed, to its position and form, to the presence or absence of albumen (which in Nymphæa is double), to the changes which take place in the integument by the absorption or thickening of the cells, &c. The contents of the cells of the albumen must be tested with reagents. The testa of the seed exhibits different

modes of cell-thickening in different plants; spiral thickenings are found in the testæ of the genus Orchis, which consist of one row of cells, and also in those of Pedicularis. Very complicated forms of this sort of thickening are met with in some Coniferæ, and the testæ of Sarisburia, Taxus, &c., exhibit woody cells.

The embryo of monocotyledons presents far more variety in its form and in the arrangement of its parts than the embryo of dicotyledons. In investigating the germination both of monocotyledons and dicotyledons, the first thing which is necessary is an accurate investigation of the embryo before germination, so as to ascertain whether the vascular bundles are then already present, and what course they take, and whether the rudiments of leaves are to be found in the plumule or not. The progress of the germ must then be watched at short intervals, and attention must be given to the growth of the axis, that is, the stem and root, as well as to the growth of the leaves, and to the distribution of the vascular bundles. Thin transverse and longitudinal sections are here necessary.

On the method of tracing the developement of the Cell.—In tracing the developement of the cell, far less skill in preparation is generally requisite than in tracing the developement of the parts of plants, but nevertheless the inquiry is a most difficult one. The reagents are in general of little service here. Iodine and sulphuric acid have too strong an effect upon very young cells. The cell-contents generally coagulate immediately. A solution of iodine, or of choride of zinc and iodine, a diluted solution of potash, or diluted acids, are preferable. Syrup and glycerine may also be used. A large number of observations is of the greatest importance.

The developement of free cells may be studied in the spores and gemmæ of Cryptogams, and in the pollen of Phanerogams. In order to examine the developement of spores and pollen, it is necessary to make thin transverse and longitudinal sections through the situs of the spores, and through the anthers, in the earliest stage of their developement. In the mosses and liverworts, the sections must be made at such a period as to exhibit only one row of the primary mother-cells in the circumference of the columella. In ferns the examination must begin at

the time of the appearance of the Sporangium in the form of a simple cell: in the Phanerogamia young anthers must be taken at the period of the appearance of a single row of mother-cells in each chamber of the anther. When the size of the rudimentary spores or of the young pollen-cells has been ascertained, their diameter measured, and their mass observed, the subsequent examination must be carried on step by step. The spike is a very favourable style of inflorescence for examining the pollen. The investigation must here be commenced with the buds of the summit of the apex, and must be carried downwards by degrees without passing over any part. In very young stages of a plant transverse sections are best made through the whole bud; the anthers which have been thus cut through must then be detached by the aid of the simple microscope, the disposition of the mother-cells in the chamber of the anther must be carefully observed, and these latter cells must then be isolated by removing the surrounding parenchyma with the needle. In examining the mother-cells, both of pollen and spores, the attention must be directed to their size and to the nature of their walls; it must be ascertained whether these walls are double or single, and the manner in which they are acted upon by iodine, and by the iodized solution of chloride of zinc, must be observed. Particular attention must also be paid to their contents. Inquiry must also be made as to the existence of a cytoblast, and whether the same is central or attached to the walls, whether it exhibits a tendency to divide, or whether division has already begun to take place; moreover, the number of its nucleoli and the distribution of the mucilage, which is usually of a granular nature, over the cell-wall, and the relation of this mucilage to the cytoblast, must also be observed. Lastly, it must be seen whether these primary mother-cells become themselves the mother-cells of others, or whether the spores and the pollen-grains are developed within them. In Anthoceros the latter is the case; in the anther of Meriolix the mother-cell forms, in the first instance, new generations of other mother-cells, before the four pollen-grains are developed, which development takes place in the last generation; the number of these generations of mother-cells can hardly be determined with

accuracy, since there is a deficiency of reliable observations relative to the successive steps of the developement. In some cases difficulties arise from the nature of the contents of the mother-cells and of the cells produced by them. These contents are often granular. Inasmuch, however, as the observations which we are now considering are carried on, not with one but with a great number of cells, those specimens can be selected which appear to be most favourable for the investigation. The use of a diluted solution of potash is sometimes advantageous. The examination must be carried on until the formation of the spore or pollen is perfect. By means of chemical reagents (such as iodine, solution of chloride of zinc, sulphuric acid, iodine and sulphuric acid, and caustic potash) the chemical nature of the cell-wall and of the cell-contents is ascertained, as well as the chemical changes undergone by the cell during its different stages of development. Alcohol, syrup, glycerine, and diluted acids often produce a contraction of the primordial utricle, and the use of these reagents must therefore not be neglected. A maceration of the whole anther or young spore, according to Schultz's method, and a subsequent treatment of them with the iodized solution of chloride of zinc, might, perhaps, be beneficial. The observations previously made with respect to the perfect flower are applicable to the treatment of the perfect spore and pollen. The developement of Gemmæ must always be traced from the primary cell, whether they be formed in peculiar organs, or at certain definite or indefinite positions on the plant. The developement of the Gemmæ of Blasia affords much interesting matter for observation; in this case thin longitudinal sections must be made through the rudimentary Gemmæ. Similar Gemmæ are developed in Winter and in Spring on the underside of the youngest leaves of Jungermannia anomala; in this case thin longitudinal sections must be made through the middle of the stem. It would also be of much importance if the Gemmæ of cryptogamous plants were examined with reference to their subsequent developement into perfect plants, and if this developement were considered in connexion with the history of the germination from the spore of plants of the same species. Although the cells of the apex of

the stem are young and in a state of active growth, they cannot be recommended for observing cell-formation in close tissues, because these cells are very small, and their granular contents render observation difficult. The leaf of Sphagnum and the leaves of liverworts are better fitted for this investigation, which, however, can be best carried on upon the cell-formation in the embryo-sac. The first cells in the embryo-sac originate around free nuclei; these first cells afterwards become mother-cells, which form secondary cells (Tochter-zellen) by division of their cell-contents. In the embryo-sac, therefore, free cell-formation and cell-formation by division may both be observed. Cynoglossum, Pedicularis sylvatica, Rhinanthus major, &c., are favourable plants for this purpose.

I believe that there is only one kind of cell-formation, that is, a formation of young cells in the interior of mother-cells. I believe that no cell-formation ever takes place without the presence of a cytoblast; the cytoblast originates in the division of an older cytoblast, which latter generally falls into two pieces. In Anthoceros the new cytoblast becomes divided in the same manner. The influence of the cytoblast upon the origin of the young cell is very clearly seen in Anthoceros. A mass of mucilaginous threads pass off from the periphery of the mother-cell to the cytoblasts; the mucilaginous investment of the mother-cell is finally divided into four parts, into four closed mucilaginous cells, each of which has its cytoblast; the layer of cellulose which is devoid of nitrogen is afterwards developed over the primary nitrogenous membrane. In the embryo-sac of phanerogamous plants also, the cytoblast appears first; this is surrounded by a more or less extensive zone of mucilage, out of which the primary nitrogenous covering, *i. e.*, the primordial utricle, appears to be formed, and over the primordial utricle the covering of cellulose, *i. e.*, the proper cell-membrane is formed at a subsequent period.

In the embryo-sac the cell-formation always commences in the periphery of its membrane; it would appear, therefore, that the nitrogenous covering of the latter, that is, the primordial utricle, is active in cell-formation. I must, therefore, in common with most vegetable physiologists, admit two modifications

of cell-formation; the one where the primordial utricle of the mother-cell divides into as many parts as there are young cells originating from the mother-cell; the other where no direct division of the primordial utricle takes place: in both cases the membrane of the cell, which consists of cellulose, is formed subsequently to the primordial utricle. The zoospores of Chlamidococcus, Ulothrix and Vaucheria, and perhaps of all Algæ, have at first no covering of cellulose. From the circumstance of the primary primordial utricle becoming divided into a definite number of secondary primordial utricles, the cellulose covering of the mother-cell appears in most cases to be expended in the formation of new coverings of cellulose for the young cells. The cellulose of the mother-cell often becomes softened to a gelatinous consistency contemporaneously with the division of the primordial utricle; in this case it forms septa clearly consisting of cellulose; at a subsequent period this cellulose entirely disappears: moreover, in every case, new cellulose for the thickening of the cell-wall is formed from the primordial utricle of the young cell. At what period this formation of cellulose takes place is a question. The first covering of cellulose which is formed round the primordial utricle of the young cell is sometimes dissolved at a later period, together with the membrane of the mother-cell. This happens in many kinds of pollen-grains. This first layer of cellulose is called by Nägeli the special mother-cell, and may be seen in the pollen of Althœa. In Ulothrix and some other Algæ, the wall of the mother-cell is not absorbed.

CHAPTER VIII.

EXAMPLES OF THE DEVELOPEMENT OF FLOWERS.

In the figures which occur in this chapter, as well as in the rest of the work, the following abbreviations are used:—

THE FLOWER.	
anth.	Anther.
bract.	Bract.
filam.	Filament.
gemm.	Ovule.
germ.	Ovary.
m. poll.	Pollen-mass.
pet.	Petal.
sep.	Sepal.
spermoph.	Spermophore.
stigm.	Stigma.
styl.	Style.

THE OVULE.	
ch.	Chalaza.
em.	Embryo.
edp.	Endosperm.
i. e.	Integumentum externum.
i. i.	Integumentum internum.
i. s.	Integumentum simplex.
nc.	Nucleus.
se.	Embryo-sac.
tp.	Pollen-tube.

As a specimen of a regular flower, that is to say, a flower in which each whorl consists of the same number of parts, I have chosen Asclepias, because the filaments, the anthers, and the ovary, exhibit peculiarities of construction arising in the course of developement.

As specimens of irregular flowers, I have chosen Stachys and Salvia, because in them the stamens are deficient in number; and Cleome, because in this flower the whorl of stamens consists of more parts than the two preceding whorls.

Asclepias Syriaca.—Fig. 63 represents the developed flower of Asclepias Syriaca, seen from above.

Fig. 63.*

Fig. 64.

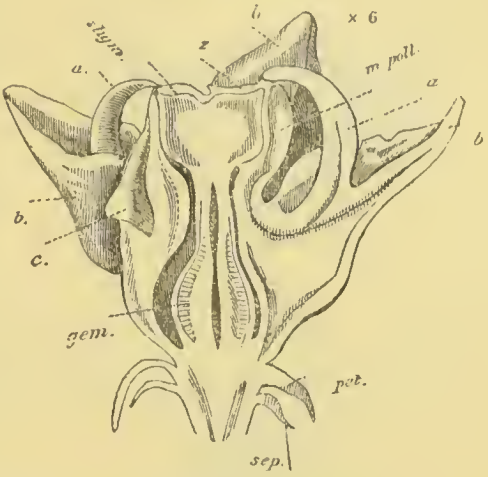


Fig. 64 represents the same flower bisected longitudinally, *a*, *b*, *c*, represent the same parts in each of the figures; *a* and *b*, are the appendages of the filament, which will be mentioned hereafter.

Fig. 65 represents the same flower seen from the side.

Fig. 65.

The first rudiments of the flower appear in the form of a small round cellular excrescence in the axil of the young bract; shortly afterwards, five small protuberances appear in a circle upon this excrescence; these are the five leaves of the calyx, *i. e.*, the sepals, and are seen in fig. 66, which represents a transverse section of the rudimentary flower in a very young state.

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* In this Figure, "× 6" implies that the flower is magnified six diameters; and so of the other figures where the sign occurs.

A longitudinal section made at this period is represented in fig. 68, and exhibits the apex of the axis in the form of a slightly-arched surface surrounded by the sepals. After a short time, the five petals appear in the form of five protuberances alternating with the sepals, which, in the mean time, have become larger; soon afterwards, a third whorl makes its appearance, consisting of five little excrescences alternating with the petals; these are the anthers, and are to be seen in fig. 69. Up to this point the apex of the axis has retained the form of an arched surface; now, however, two small protuberances proceed from it, which are the first rudiments of the ovary. These two small protuberances are seen in fig. 69 within the anthers.

Fig. 67.

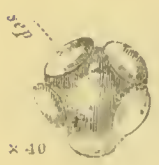
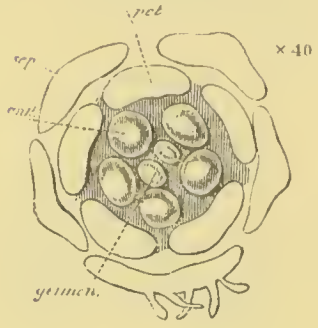


Fig. 68.

Fig. 69.



All the parts of the flower are now established, and continue to be developed together. There is nothing further requiring particular notice in the calyx and petals; the five stamens and the two pistils now alone arrest our attention. The anthers, which, in their origin, were in the shape of round excrescences, appear soon afterwards, upon making a transverse section of them, to be elongated, and to be of the form shown in fig. 70, which represents a transverse section of a more advanced flower, three of the sepals being omitted. The longitudinal section, fig. 71, shows that they are inserted somewhat higher up than the

Fig. 70.

petals. If a transverse section be made shortly afterwards, the vascular bundle of the connective may be seen. Fig. 72 represents such a transverse section, the sepals being omitted,

Fig. 71.

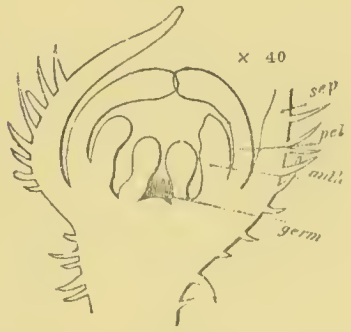
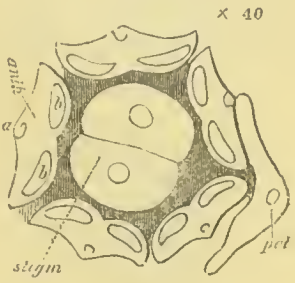


Fig. 72.



and only one petal drawn; (*a*) represents the vascular bundle, and (*b*) (*b*) the rudiments of the two loculi of the anthers. Figs. 73 and 74 represent longitudinal sections, which also show

Fig. 73.

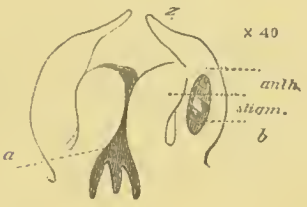
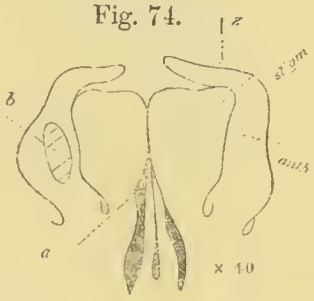


Fig. 74.



at (*b*) in each figure, the origin of the loculi of the anthers; the apex of the anthers (*z*) is spread out, as appears in both figures, flatly over the stigmas; the filament of the anther is as yet simple without any appendages. The sepals and petals are omitted in figs. 73 and 74.

Let us now turn to the pistils, which we left at fig. 69, in the form of two small wart-like excrescences surrounded by the anthers. In fig. 70 we have seen them as two half-moon-shaped organs with their edges turned towards each other. Fig. 71 exhibits a longitudinal section of them answering to the transverse section in fig. 70. As the developement proceeds, the curvature of each rudiment of the ovary increases, the two edges approximate more and more at their base, and at last

turn completely inwards; the upper parts of the pistil on the other hand do not become curved in one mass with the ovaries; in fact, the stigma does not become curved at all; the style, therefore, does not terminate as in other plants at the apex, but underneath the stigma.

The longitudinal sections, figs. 73 and 74, exhibit at (*a*) the place where the style ends; at a later period there is formed there a conducting tissue consisting of long papillæ. Fig. 75 represents a longitudinal section through the upper part of the pistil of a flower almost developed; (*a*) is the place under the stigma where the pollen-tubes enter the canal of the style; (*y*) is the secreting portion of the stigma, and will be referred to hereafter. The figure only shows half the pistil. In figs. 73 and 74 the stigmas of the two pistils are not yet grown together; a little later they become united, as shown in fig. 76, which represents a longitudinal section with the calyx and petals removed, and showing only one anther. Transverse sections through the ovaries made at this period at different heights, as, for instance, at the points *a' b' c'* and *d'* in fig. 76, exhibit the circumstances which have been heretofore stated with respect to the developement of the rudiments of each individual ovary. Fig. 77 (*a'b'c'd'*) represents four transverse sections through the two ovaries of a young flower, at different heights, such heights being shown in fig. 76 by the same letters of reference; *d'* is the lowest part of the two young pistils, + + is one of the vascular bundles at the bottom of the

Fig. 75.

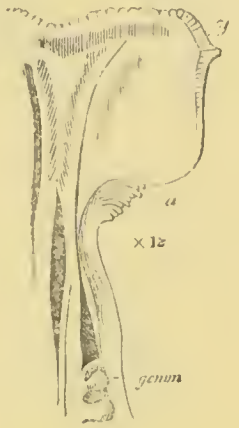
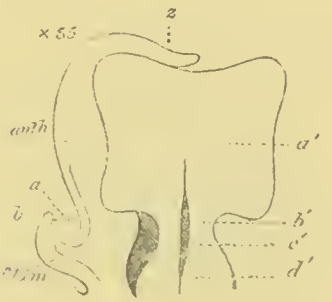
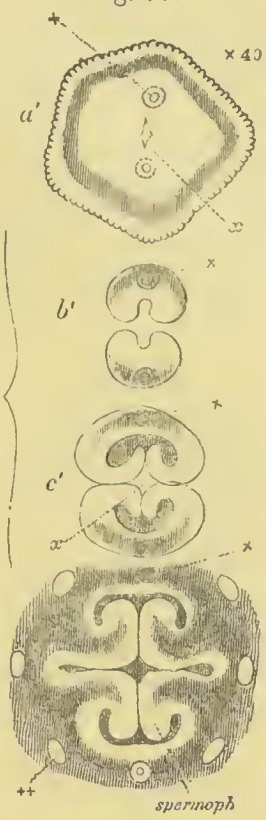


Fig. 76.



flower; ×, on the other hand, which recurs at *c′ b′* and *a′*, is the vascular bundle of the pistil itself. At *d′*, in fig. 18, the two edges of each pistil turn completely inwards; at a later period they form the placentæ, shown in fig. 78, which represents a transverse section of the lower part of an ovary at the time of flowering; at *c′*, in fig. 77, the curvature is still present, and through it the canal of the style (*x*) takes its origin; at *b′* in fig. 77, the end of this canal is seen underneath the stigmas, and at *a′* the last trace of the canal of the style (*x*) is seen within the two stigmas which have now grown together. About the time when the two stigmas grow together a slight excrescence arises underneath the anthers, which are now to a certain extent developed; in the axil of this excrescence a small protuberance arises (*see* fig. 76, *b*, *a*). At this spot a peculiar bending in the vascular bundles of the anthers takes place, which bending increases perceptibly with the further development of the appendage above mentioned.

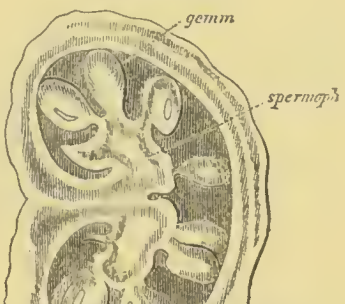
Fig. 77.



(*a*) and (*b*) fig. 76, subsequently become developed into (*a*) and (*b*) figs. 63 and 64.

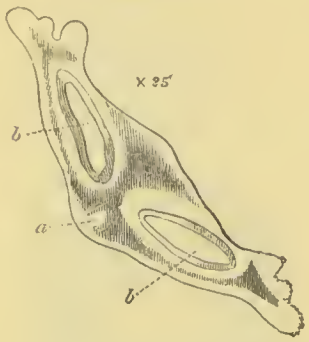
We have now reached the full development of the flower; the calyx and petals are not grown together, but bend downwards at the time of flowering (figs. 64 and 65); the filaments of the

Fig. 78.



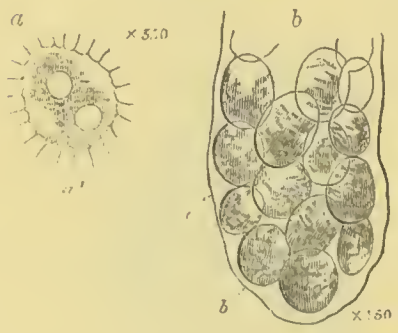
anthers, on the other hand, are joined one with another at their lower extremities to a fleshy ring; besides the two appendages above-mentioned (*a* and *b*, fig. 64), there appears on each side of the anthers a further wing-shaped expansion of the filament; in fact, two wings, belonging to different filaments, are placed close to one another, figs. 63 and 64 (*c*), whilst a thin, skin-like expansion (*z*), (figs. 63, 64, 73, 74, 76) proceeding from the apex of the anthers, covers the stigma. The anther is from the first bilocular (fig. 72, and fig. 79). Fig. 79 represents a transverse section of the upper part of a perfect anther.

Fig. 79.



Owing to the peculiar form of the stamens, very different appearances are presented, according to the height at which such a section is made. (*a*) is the vascular bundle of the connective (*b*), (*b*) the two chambers of the anther; no separate pollen-grains are developed in the two loculi, as is the case with most other plants, but they each contain a pollen mass covered with a leathery skin. I was unable to follow the developement of the cells of these pollen-masses with sufficient accuracy, on account of the granular nature of their contents; the mother-cells at a later period lie in rows (fig. 73 (*b*), and fig. 74 (*b*)). In fig. 80 at (*a'*) the primary mother-cell is represented, as it appears in a transverse section through a very young anther surrounded by the small adjoining cells; two cytoblasts, and a line between them, show the origin of two new cells in the mother-cell. In fig. 80 (*b'*) is shown part of a thin transverse section through the perfect pollen-mass, (*a*) being the leathery skin surrounding the pollen-cells (*b*). I consider the

Fig. 80.



leathery skin to be a secretion; it becomes of a rich claret colour upon the application of concentrated sulphuric acid; the substance which is diffused here and there through the pollen-cells becomes coloured in like manner.

The stigma is pentagonal: at five particular points of it the epidermis is developed into the form of papillæ (fig. 75 *y*). These places which, in a transverse section, assume the form of channels, secrete a fluid which gradually hardens, assumes a definite form, and becomes converted into the so-called gland, which unites the two pollen-masses.

Fig. 81.

In fig. 81 is represented a small part of a thin transverse section through the stigma of a flower almost developed; *y* is the secreting epithelium, consisting of long, thin papillæ; *x* is the hardened secretion, or so-called gland (also in transverse section), and which is united to the papillæ. Fig. 82 represents two pollen-masses belonging to different anthers united by the so-called gland (*x*) and by the caudiculi.

Fig. 82.

The secreting surface of the stigma is extended in a modified form as far as the place where the chamber of the anther opens: the secretion from this surface forms the caudiculus which is protruded by the so-called gland, and which bears the pollen-masses. The position of the secreting surface of the stigma at (*c*) (fig. 63), gives rise to the peculiar circumstance that each one of the so-called glands unites two pollen-masses, produced from two different anthers.

In tracing the developement, it appears most clearly that that which has been called a gland is not really a gland, but is a true secretion. Upon making very thin transverse sections through the stigma at different periods of its growth, the secretion will be found to be at one time fluid, and at another half fluid, and already hardened on the outside, whilst additions are

made to it by new secretions from the surface of the stigma itself. The apparently cellular structure of the complete mass is caused by the impression of the secreting cells; the uniform nature of this mass is seen by making a thin section through it, and treating it with caustic potash. The so-called gland of the Asclepiadeæ is therefore something quite different from the retinaculum of the Orchideæ, which really consists of cells containing a slimy glutinous fluid.

The Asclepiadeæ, as was known long since, can only be impregnated by insects, or artificially. The place where the pollen-tubes can penetrate lies close under the stigma, fig. 75 (*a*), and is shown by long papillæ; the epithelium of the filament of the anthers also forms a secretion at this place. The two ovaries of the perfect flower remain completely separated down to their base, although their stigmas adhere to one another; upon making a tranverse section, the placentæ of each ovary appear spread out on both sides, and bearing many rows of ovules, as represented in fig. 78.

Fig. 83.

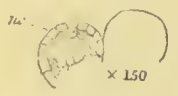


Fig. 84.

Fig. 85.

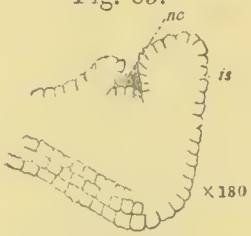
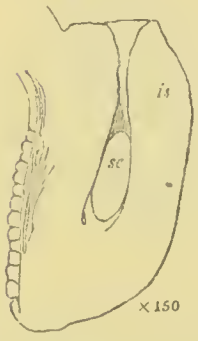


Fig. 86.



The ovule has only one integument, and the nucleus, which is but slightly developed, disappears at an early period, being absorbed by the embryo-sac. It is not visible at the time of flowering. Figs. 83, 84, 85, and 86, represent different stages of the developement of the ovule.

The pistil increases in growth at its apex; the two stigmas are formed last; they are originally separate, and become united at their apex at a subsequent period, so that the growing-cells must be situated at their apex (fig. 74 and fig. 76). The anthers, on the other hand, grow at their base; the very

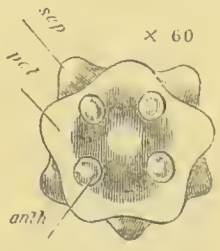
beautiful appendage (*z*) is already present when the chambers of the anthers are forming (figs. 73, 74, 76, and 64). The appendages (*a*) and (*b*) of the filaments, on the other hand, appear at a much later period; the increasing curvature of the vascular bundle, which takes place contemporaneously with the further developement of the above-mentioned appendages, indicates a further developement of the vascular bundle at this place.

The two stigmas of the separate pistils are united by a real *adhesion*, or *growing together*. In other plants, the parts which appear to have become grown together, have not really become so, but only apparently, the fact being that the original separation of the parts is not continued in their subsequent growth.

Stachys Coccinea.—The rudiment of the flower appears, as in the case of Asclepias, in the form of a small cellular cone in the axil of the bract. A short time afterwards five protuberances appear upon it, which are the five sepals. Fig. 87 represents a longitudinal section of the first rudiments of the flower. At a later period a second circle, consisting of similar excrescences, is perceived; these are the petals, which alternate with the sepals. A third whorl now follows, which, however, consists of only four elements, alternating with the rudiments of the petals. These are the anthers, the fifth of which has not begun to be developed. Fig. 88 represents this stage of the flower seen from above. It is very probable, notwithstanding the pains I have taken, that that condition of the plant which would exhibit the rudiment of the fifth anther arrested in its growth, may have escaped my attention; in Salvia nivea I was so fortunate as to meet with the three arrested anthers in their rudimentary state. Fig. 89 represents a transverse section of a very young bud of Salvia nivea (*x*), being the traces of the three abortive anthers. The parts of the calyx in Stachys coccinea, as well as of the

Fig. 87.

Fig. 88.



corolla, are separated at first; they do not afterwards grow together, but the fact rather is, that the separation ceases during their subsequent developement. In fig. 88 we have seen the petals united at their base. At a somewhat later period the parts of the calyx, as well as of the corolla, are completely united to one another; that is to say, the lower portions of them have never been divided into separate parts from the first period of their growth. The growth of the calyx and corolla is here shown to be analogous to that of the leaves, in the fact that the divided apices have been first formed.

Fig. 89.

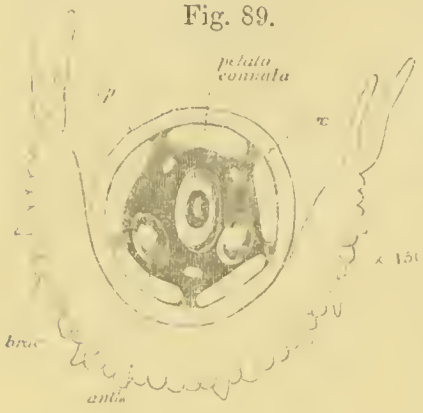


Fig. 90 and fig. 91 are transverse sections of one and the same flower at different heights; at fig. 91 is seen the empty situs of the fifth abortive anther. The ovary here does not consist of leaves originally separate and afterwards grown together; it rather appears to be always one and entire. The anthers are at first inserted only a little above the petals, as is seen at fig. 92, which represents a longitudinal section of the flower at a period somewhat later than that represented in fig. 90 and fig. 91. The ovary of the Labiatæ as well as of the Boragineæ appears, by following out its developement, to be uni-locular, with two parietal placentæ, each of which bears two ovules. Fig. 93 represents a transverse section through a young ovary. From the subsequent unequal developement of the wall of the ovary, which appears to be pro-

Fig. 90.

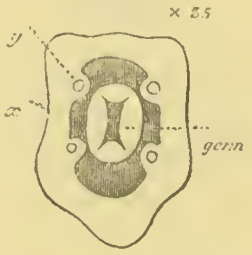
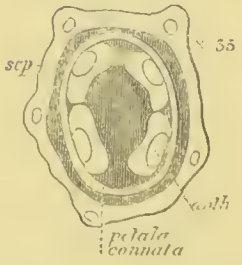


Fig. 91.



duced by the perfecting of the four ovules, the four nut-like excrescences originate, which are connected together by the single canal of the style. Fig. 94 represents a longitudinal

Fig. 92.

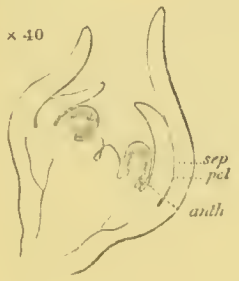


Fig. 93.

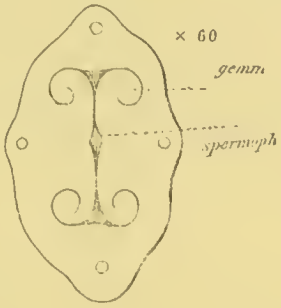
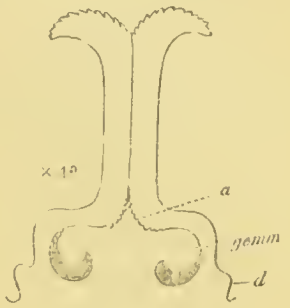


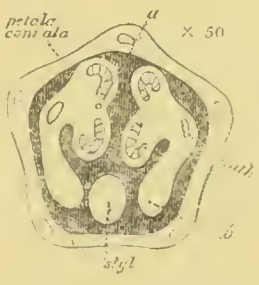
Fig. 94.



section through the young ovary with the style and stigma; (*d*) is the disc. The ovules of the Labiatæ have only one integument. In Salvia and Galeopsis I found prolongations of the embryo-sac similar to those in Lathræa and Pedicularis.

Salvia Nivea.—The first appearances of the developement of the flower are just the same as in Stachys, the calyx appears first, afterwards the petals, and then the anthers. Fig. 89, p. 168, shows a very successful transverse section through the rudiments of the flower, whilst it is still concealed by the bract; the sepals are no longer separate; the petals, on the other hand, are still separate; only two of the anthers are as yet visible in the form of large round warts; the three others appear like very small oblong excrescences (*x*): the position of these five anthers, both of the three abortive ones, and of the two which are developed, alternates with the petals; the two anthers which are not abortive produce pollen only on one side. Fig. 95 represents a transverse section of the young flower with the bract and calyx removed; the side of the anthers which produces pollen is bilocular; it bursts, when ripe, with a

Fig. 95.



longitudinal dehiscence. Figs. 96 and 97 represent the stamens in process of developement; fig. 98, the two stamens of the developed flower.

Fig. 96.

Fig. 97.

Fig. 98.

Cleome Arborea.—The first rudiments of the flower appear, in the usual manner, in the form of a cellular cone in the axil of the bract; shortly afterwards appear the rudiments of the four sepals, and next to these come the four petals alternating with the sepals. Figs. 99 and 100 represent a flower in a young state seen from above. After this, however, follows a whorl of six elements. Fig. 101 shows a flower at a somewhat later stage than is represented at fig. 100. In fig. 101 the sepals are removed, and only two of the petals drawn. Fig. 102 represents a very perfect transverse section, showing all the parts of the flower. It might, perhaps, be supposed that there were two whorls of anthers, each whorl containing four elements, and that two of the elements of these whorls were abortive; but that this cannot be so is shown by the regular position of the six anthers in one whorl, which always occurs in good specimens. The long-stalked ovary, which is subsequently developed, appears in the form of a solid column; at its apex there is formed a small depression, which at first is very flat; the depression subse-

Fig. 99.

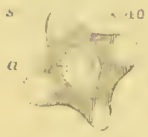


Fig. 100.

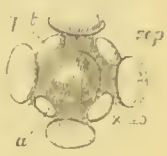
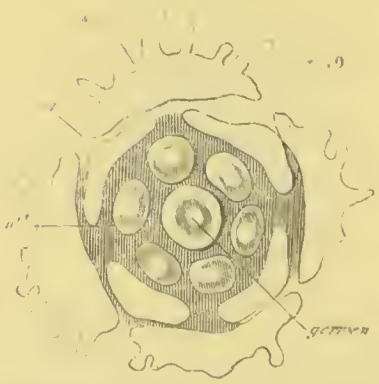


Fig. 101.

Fig. 102.



quently increases, and the ovary assumes the form of a cup. The edge of this cup afterwards increases in thickness, its walls approximate to one another, and form the stigmas and the style. The ovary is unilocular with two parietal placentæ. The ovule has two integuments; at a later period it exhibits a peculiar curvature; the anther is quadrilocular, but at the time of dehiscing it is bilocular.

Fig. 103 represents a longitudinal section corresponding to the stage of development, represented at fig. 102. Figs. 104 and 105 represent longitudinal sections of the ovary in different stages

Fig. 103.

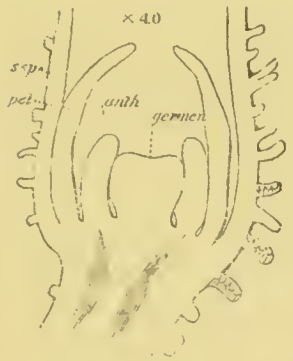


Fig. 104.

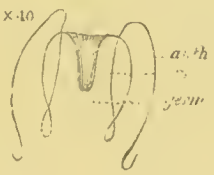


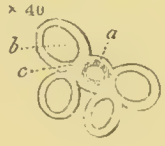
Fig. 105.

of development, the sepals and petals being removed; (*x*) is the hollow of the ovary. Figs. 106 and 107 represent transverse sections of the ovary in different stages of development. Fig. 108 represents a transverse section of the young anther; it

Fig. 106.

Fig. 107.

Fig. 108.



is quadrilocular; (*a*) is the vascular bundle of the connective; (*b*) one of the chambers of the anther; (*c*) the cellular tissue of the connective, which is absorbed shortly before the dehiscence of the anther.

CHAPTER IX.

ON THE STRUCTURE OF THE POLLEN OF THE CONIFERÆ.

The existence of naked ovules constitutes one essential mark of distinction between the Coniferæ and Cycadeæ and all other phænogamous plants, but a more important difference is to be found in the manner in which the rudiment of the embryo is developed in the interior of the ovule. It is therefore not surprising that the pollen which plays so important a part in impregnation should, in these plants, present extraordinary peculiarities. In all other cases the pollen-tube makes its appearance in the form of a tubular prolongation of the innermost pollen-cell, whilst in all the Coniferæ which I have examined, a body consisting of several cells is formed within the pollen-grain, and the terminal cell of this body displaces the contents of the real pollen-cell, and eventually forms the pollen-tube.

Fritsche, in his treatise upon Pollen, noticed the existence of these cells in the interior of the pollen of the Coniferæ, and they have been more fully and accurately figured and described by Meyer in his "Vegetable Physiology." At p. 188 of the third volume, Meyer says, "The pollen-grains of Larix are generally round, they exhibit a delicate outer membrane, a middle membrane of greater thickness which is gelatinous and transparent, and an inner membrane; at a certain spot, between the outer and the middle membrane, there is to be found a narrow fissure, which under a low magnifying power appears only as a dark streak. Immediately under this fissure is a small cell, which is fastened to the middle membrane, and filled with a granular substance, and which serves as a pedicel for a larger cell in the interior of the pollen-grain."

Meyer found in the pollen of Pinus sylvestris and Picea vulgaris a small body precisely similar, consisting of two cells, a

smaller one and a larger one; and he states that he has also observed in certain Liliacæ from one to three cells, not united together, but lying free in the interior of the pollen-cell. Meyer considers all these cells to be imperfectly formed organisms. The same subject was afterwards more fully discussed by Geleznoff (Annales des Sciences Naturelles, 1850), who has furnished a history of the developement of the pollen as well as of the embryo in the larch. There are, however, in my opinion, some defects in his account of the developement of the pollen-grains and of the cellular body in their interior. Geleznoff (with Fritsche) assumes the existence of four pollen-membranes; according to his view, the fourth or innermost membrane is formed from the terminal cell of the cellular body, which gradually displaces the contents of the true pollen-cell, *i. e.*, of the third membrane. The pollen-tube of the larch consists, according to Geleznoff, of two membranes, namely, of a prolongation of the true pollen-cell, and a contiguous prolongation of the terminal cell of the cellular body. The small cells constituting the pedicel of the cellular body are very accurately represented. The pollen-tube, consisting of two membranes, is now supposed to penetrate into the interior of one of the corpuscula of the embryo-sac, and to remain at its apex, whilst the inner membrane, *i.e.*, the prolongation of the terminal cell of the cellular body, makes its appearance, and forms the embryonic vesicle, that is to say, the first cell of the embryo. The investigations of Geleznoff, therefore, confirm Schleiden's theory of impregnation, according to which the first cell of the embryo of the Coniferæ originates in the pollen-tube itself.

The bud of the male catkin of Abies pectinata makes its first appearance in Spring, at the time of the opening of the branch-bud, in the form of a small green protuberance in the axis of a leaf, and on the under-side of the young branch. These buds are found in large numbers upon every terminal branch which has borne male flowers during the current year, which flowers usually open earlier than the branch-buds. The rudiments of the catkin-bud are probably formed in autumn, soon after the origin of the leaf in the axil of which it appears, and in the Spring of the first year it consists of a *punctum vege-*

tationis, protected by a few scale-like leaves; until the middle of the Summer it continues to form scales only. It is not until the young branch upon which the bud is placed has attained its full growth, and has begun to form its autumnal wood, that the *punctum vegetationis* elevates itself, and from that time no more scales are formed, but rudiments of leaves begin to grow, which by degrees become developed into anthers.

The catkin-bud of Abies pectinata only differs at first from the cone-bud of the same tree, in being situated on the underside of the young branch, and in the fact of the contemporaneous appearance of many buds in the neighbourhood of one another. The cone-bud always appears on the *upper* side of other (female) branches, and is, moreover, solitary. The process of developement which takes place in the interior of both species of buds is at first alike; in the latter part of the Summer, however, as soon as true leaves are formed in lieu of scales, essential differences arise: the leaf of the male catkin exhibits no axillary buds, which latter are found in the cone-bud shortly after the appearance of the leaf, and these in the succeeding Spring form fruit-scales, upon which the ovules are developed. On the other hand, the leaf of the male catkin becomes the anther, whilst a longitudinal row of large cells is developed on both sides of a *cambium-string*, which originates from the thickening-ring of the axis or stem of the bud. These cells are nourished by the contiguous smaller cellular tissue, and in their interior the mother-cells of the pollen-grains, or the cells which give origin to the mother-cells, are formed.

The vascular bundle, or connective of the anther, is formed in the following year out of the cambium-string. Even in August the young anther of Abies pectinata, Picea vulgaris, Pinus sylvestris, and Larix is bilocular. By the end of September, four young pollen-grains are found in each mother-cell of these anthers. From this time until the beginning of Spring, no changes of importance take place; the cone-bud also makes no further progress during the Winter. At the beginning of May, the pollen-grains of Abies pectinata and Picea vulgaris lie in fours in the interior of their mother-cell.

Fig. 109 represents a young pollen-grain of Abies pectinata seen in water, and fig. 110, a somewhat older pollen-grain, also seen in water, where (x) is the place of the thin spot in the euticle.

Fig. 109. Fig. 110. Fig. 111.

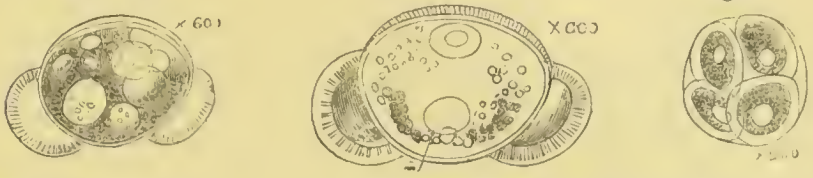


Fig. 111 represents a mother-cell of Abies pectinata with four young pollen-grains. At the beginning of May the pollen-grains of Picea vulgaris, if placed in water upon the stage of the microscope, frequently swell up and escape from the mother-cell; when this takes place, cells called special mother-cells (which exist also in certain other plants, as, for instance, Lavatera) are found to be present.

Figs. 112 and 113 represent mother-cells of Picea vulgaris, which have become quadrilolucar by the formation of special mother-cells; the young pollen-grains having been swollen by water have escaped from the mother-cells. These special mother-cells, which were first observed by Nägeli, are nothing more than a primary layer of cellulose secreted by the primordial utricle of the pollen-cell, the pollen-cell itself having originated by division of the primordial utricle. This layer of cellulose, like the mother-cell, is afterwards either absorbed by the pollen-grains themselves, or appropriated in some other way to the perfecting of them. When the four young pollen-cells of Picea escape from their mother-cells, the latter appear, to a certain extent, to be divided by the special mother-cells into four partitions. (*See* fig. 113.) The young pollen-grain is then generally no longer round; the two lateral excrescences, which at a subsequent period characterize the pollen-grain of Picea, Abies, and Larix, are already more or less perceptible.

Fig. 112. Fig. 113.

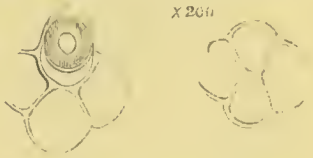
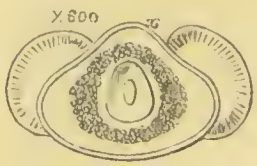


Fig. 114. Fig. 115.

Figs. 141 and 115 represent young pollen-grains at this

period, shewing the commencement of the formation of the two lateral excrescences. Fig. 116 shews a young pollen-grain of Picea vulgaris, somewhat further developed, seen under water; (x) is the place of egress of the pollen-tube. A large nucleus, surrounded by granular matter, lies apparently free in the middle of each pollen-grain. From this time the lateral protuberances continue to increase in size, the cuticle, *i.e.*, the outer membrane of the pollen-grain, which does not consist of cellulose, becomes stronger and stronger, and delicate markings, streaks and lines appear upon it, especially upon the protuberances.

Fig. 116.



Figs. 117 and 118 represent respectively ripe pollen-grains of Abies pectinata and Picea vulgaris which have been soaked

Fig. 117.

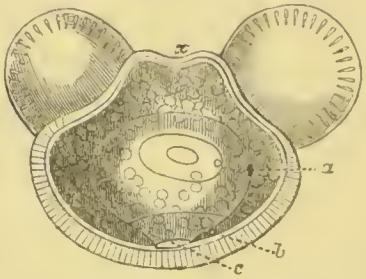
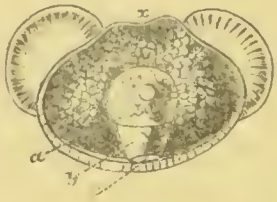


Fig. 118.



for half an hour in oil of lemons; in each figure (a) is the terminal cell of the cellular body; (b) and (c) the pedicel-cells: in both (c) has the appearance of a fissure, as is the case in Larix; (x) is the place of egress for the pollen-tube. The processes going on in the interior of the pollen-grain are at this time concealed, more or less, by the granular contents: nevertheless, I believe that in Abies I have seen the formation of a cell-membrane, that is the origin of a cell, around the central nucleus. When the anther of the above-mentioned Conifers opens, which takes place about the end of May or the beginning of June, every perfect pollen-grain of Larix Europæa, Abies pectinata, Picea vulgaris, and Pinus sylvestris, exhibits the cellular body mentioned by Meyer; there may also be seen the fissure between the outer and the inner membrane, *i.e.*, between the pollen-cell and the cuticle; the latter seems to consist of

two layers, on which account Meyer speaks of three pollen-membranes. There is also to be seen above this fissure the small pedicel-cell, and over this again a larger cell, which encloses a very manifest large nucleus. Fig. 119 represents a ripe pollen-grain of Larix Europæa seen in water; (*a*) is the terminal cell of the cellular body; (*b*) the larger pedicel-cell; (*c*) and (*d*) the smaller pedicel-cells, having the appearance of fissures in the membrane of the pollen-grain. In Larix the cellular body seems frequently to be composed of four cells instead of three. (*See* figs. 117 and 118.) The above-mentioned cellular body lies opposite to either side of the arcuate space which is found between the two lateral excrescences, and out of which at a later period the pollen-tube emerges. At this place there is to be found in the cuticle of Abies pectinata an attenuated spot, which is rendered manifest in favourable positions of the pollen-grain by the use of sulphuric acid; the same kind of spot probably exists in Picea vulgaris and Pinus sylvestris, and it may be seen also in the cuticle of the Larch, in which plant also the cellular body is situated opposite to this place of egress of the pollen-tube. According to Gelcznoff, both the outer membranes, and therefore the cuticle, are completely stripped off. Fig. 120 represents a ripe pollen-grain of Larix Europæa under concentrated sulphuric acid. The contents of it, as well as the true pollen-cell, have disappeared; some oily drops (*y*) are to be seen in the middle of the cuticle (*ct*), which is uninjured, but which has become rose-coloured, and now exhibits two layers; (*x*) is the thin spot in the cuticle intended for the egress of the pollen-tube. The above-mentioned cellular body may be seen most clearly and beautifully in the pollen of the Larch, which is round or nearly so, and in which, as is well known, the two lateral excrescences are wanting; but neverthe-

Fig. 119.

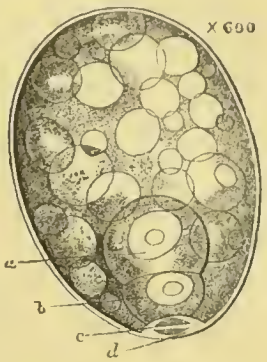
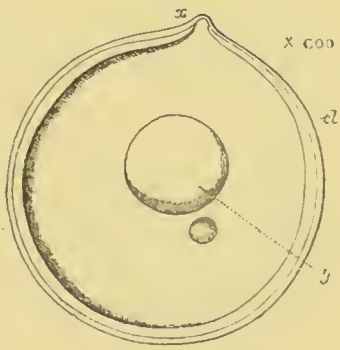


Fig. 120.



less I have derived more information from the study of the pollen-grain of Abies pectinata, Picea vulgaris, and Pinus sylvestris, for which it is necessary to make use of acids and volatile oils.

By means of the continued action of oil of lemons or oil of turpentine, the contents of the above-mentioned pollen-grains are rendered so transparent that the construction of both the small pedicel-cell, and of the larger terminal cell, as well as their attachment to the wall of the pollen-cell, may be clearly seen. (*See* figs. 117 and 118.) This mode of observation, however, affords no information with regard to the fissure underneath the pedicel-cell. If, however, one or two drops of common sulphuric acid be applied to the pollen when quite fresh, and a thin glass cover be placed over it, the immediate appearance of the pollen-cell may be observed at the spot destined for the egress of the pollen-tube; it swells and protrudes itself more or less rapidly, and in a more or less perfect condition, through the cuticle, and lies exposed before the eyes of the observer.

Fig. 121.

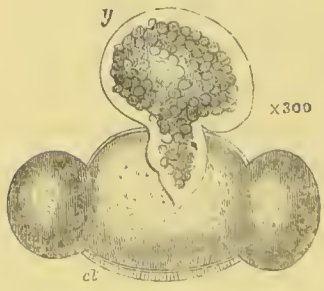


Fig. 121 represents a ripe pollen-grain of Picea vulgaris under nitric acid, at the moment when the true pollen-cell (*y*) emerges at (*x*): (*ct*) is the cuticle shewing two layers. Fig. 122 represents a similar pollen-grain under concentrated sulphuric acid; the pollen-cell and its contents have disappeared, leaving only some drops, probably of oil, behind in the cuticle. Figs. 123 and 124 represent the pollen-cell of two ripe pollen-grains forced out of their cuticle by

Fig. 122.

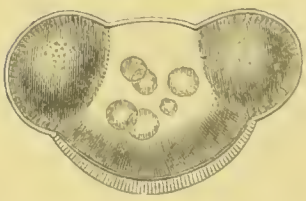


Fig. 123.

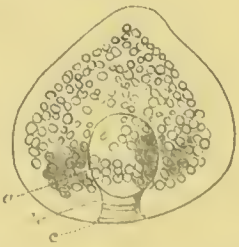
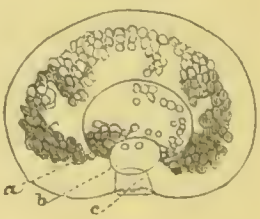


Fig. 124.



the operation of nitric acid; (*a*) (*b*), and (*c*) are the cells of the cellular body.

The empty cuticle now exhibits its markings still more beautifully. It is now seen that as well in Abies pectinata as in Picea vulgaris and Pinus sylvestris, the cuticle is very strongly developed at the two excrescences, and still more so between them, opposite to the place of egress of the pollen-tube, at the point of attachment of the cellular body. At the places where the cuticle is thus strongly developed two layers may frequently be clearly seen. The fissure between the pollen-cell and the cuticle has now disappeared; but three cells (and not two only, as in the earlier stages) are always to be seen in the emergent pollen-cell, and these three cells form the cellular body in its interior. (*See a, b,* and *c,* figs. 118, 123, and 124.) The lowest of these cells (*c*) is generally the smallest, and becomes united in its growth to the wall of the pollen-cell, which fact is clearly seen by pushing the covering-glass backwards and forwards; it was this cell which at an earlier period formed the apparent fissure between the cuticle and the pollen-cell. The contiguous cell (*b*), which is somewhat larger, and which has been hitherto called the pedicel-cell, supports a third cell, viz., the the terminal cell of the cellular body (*a*). I have never seen *more* than three cells in Abies pectinata, Picea vulgaris, and Pinus sylvestris; but I have never failed to see any one of these three cells in the perfect pollen-grain.

Although I have not been able to trace the developement of the cellular body, it may perhaps be assumed that its three cells originate from the one cell which I have observed in the pollen-grain of Abies pectinata, and probably by repeated divisions of the primordial utricle. The two pedicel-cells, when once formed, do not seem to increase in size, but the terminal-cell grows visibly: in Pinus sylvestris I have met with instances in which the latter cell has completely displaced the granular contents of the pollen-cell, and become distended to such a size as to fill up completely the hollow of the latter. The true pollen-cell appeared about this time to be much decayed, and of a gelatinous consistency; at the apex it was completely absorbed. I cannot, therefore, at present subscribe to Geleznoff's opinion, that the pollen-tube is formed of two membranes.

Fig. 125 represents a ripe pollen-grain of Pinus sylvestris viewed dry; at (*x*) the place of egress for the pollen-tube, it is

Fig. 125.

Fig. 126.

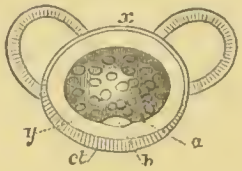
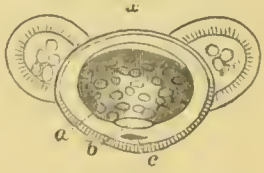


Fig. 127.

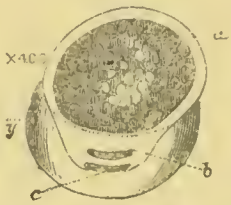


folded together. Figs. 126 and 127 represent similar pollen-grains after long soaking in oil of lemons: (*a*) (*b*) and (*c*) are the cells of the cellular body, which has already displaced the contents of the pollen-cell; the latter is gelatinous and distended: (*x*) is the place of egress for the pollen-tube.

Figs. 128 and 129 represent the pollen-cells of two ripe pollen-grains of Pinus sylvestris forced out of the cuticle by nitric acid; (*a*) (*b*) and (*c*) are the cells of the cellular body; (*y*) is the swollen wall of the pollen-cell.

Fig. 128.

Fig. 129.



I have observed the terminal cell of Pinus sylvestris, which at a later period makes its appearance as a pollen-tube, to be in some cases filled with granular matter, corresponding chemically to the earlier contents of the pollen-cell; the two pedicel-cells then appeared to be in a dead or dying state. In Picea vulgaris, Abies pectinata, and Larix Europæa, I have not been so fortunate as to be able to trace fully the subsequent developement of this terminal cell into the pollen-tube. From the results of prior observations, it seems to me to be not improbable that in other Coniferæ besides the Larch, the cuticle is completely stripped off by the tubular prolongation of the pollen-cell. In the Larch this fact has already been observed by Geleznoff.

In the Autumn of 1853, Encephalartos Altensteini produced two male blossoms at Hamburgh. I examined the pollen whilst fresh, but did not discover the above-mentioned cellular body. The pollen of the Cycadeæ is round, and is, like that of the Coni-

feræ, furnished with only one place of egress for the pollen-tube. The pollen of Encephalartos, when dry, is folded. In that which I examined, I did not meet with the granular contents which in the Coniferæ are coloured yellow by iodine, and which are made to burst, or become transformed into oil-drops, on the application of sulphuric acid. This pollen seems to me, therefore, to be abnormal, and, consequently, I do not attribute any importance to the circumstance of the non-existence of the cellular body. It remains, therefore, to be ascertained by further examinations of the pollen of the Coniferæ and Cycadræ, whether the abovementioned cellular body in the interior of the pollen is common to all the plants of these families; and whether the terminal cell of this body is intended in all cases to serve the same purposes as in Pinus sylvestris. If it be so, a new and important characteristic of these families will have been obtained. The examination of the pollen should, however, be made only whilst it is fresh; after it has become dry, the *fissure* to which I have referred may still be seen, but not the cellular body. Gelcznoff's careful observations on the impregnation of the Larch deserve to be carefully repeated.

If, as may confidently be expected, the appearances which have been here described are found to occur in all the Coniferæ and Cycadeæ, the reproductive organs and the formation of the embryo of these plants will differ from those of all other phanerogams in the three following essential particulars.

1. The Coniferæ and Cycadeæ have naked ovules, that is to say, their ovules originate in an open fruit-scale, whilst in all other (phanerogamous) plants they make their appearance in the interior of a special organ, that is in the hollow of the ovary.

2. The embryo-sac of the Coniferæ and Cycadeæ produces *corpuscula*, which are large cells of the albumen, varying in number, and situated at the apex of the embryo-sac, into which the pollen-tube enters, in order that after having extended itself within the *corpusculum*, it may form the first cells of the embryo. In all other plants the *corpuscula* are wanting, the pollen-tube simply enters into the embryo-sac, and forms therein the first cells of the embryo.

3. The pollen-tube of the Coniferæ and Cycadeæ is not (as is the case in other phanerogams) a prolongation of the inner true pollen-cell, but *an extension of the terminal cell of a small body consisting of several cells*, which body originates in the interior of the pollen-cell, the contents of which it appropriates to the purposes of its own developement.

CHAPTER X.

FURTHER OBSERVATIONS UPON THE STRUCTURE OF THE POLLEN OF THE CONIFERÆ.

In the spring of the present year, 1854, further observations afforded me the following results, which are supplementary to those contained in the last chapter.

1. In the pollen-grain of the Larch, shortly before the dehiscence of the two loculi of the anthers, the pollen-cell becomes divided, so that it falls into two unequal halves. The lesser derivative cell lies opposite to the fissure in the cuticle destined for the egress of the pollen-tube. This cell again becomes divided, and the division is generally repeated in both the derivative cells of the second order. In this manner the cellular body originates in the interior of the true pollen-cell, the wall of which is now softened to a gelatinous consistency. The two lowermost cells of the cellular body then undergo no further developement, and their contents disappear; when the pollen is shed they form the two fissures seen in fig. 119. The two upper cells of the cellular body, on the other hand, continue to grow; the uppermost or terminal cell becomes the pollen-tube, which destroys the contents of the larger primary derivative cell, and breaks through it. By this means the cuticle is stripped off in the form of a covering with two flaps. The repeated division by which the cellular body in the pollen-grain of the larch originates, generally takes place horizontally, that is in a direction parallel to the direction of the primary division. Exceptions, however, to this rule are not unfrequent, in which the subsequent division takes place in a direction perpendicular or oblique to that of the first.

2. In those Coniferæ the anthers of which are provided with pollen-sacs (Cupressus, Thuja, Juniperus and Taxus) a similar

division of the pollen-cell into two unequal halves takes place in the round pollen-grain. Here also that one of the derivative cells which lies opposite to the fissure in the cuticle is much smaller than the other. The smaller of these cells, however, does not, as in Larix, Pinus, Picea, and Abies, become developed into the cellular body which is nourished by the larger derivative cell of the pollen-grain. The smaller cell undergoes no further change; the larger cell, on the other hand, becomes the pollen-tube, which breaks through the now-softened gelatinous membrane of the true pollen-cell, whereby the cuticle, as in Larix, is stripped off in the form of a two-flapped covering. In these plants those pollen-tubes which have penetrated into the nucleus of the ovule always have their cuticle stripped off; they terminate on their free side with the small undeveloped derivative cell (Thuja, Juniperus, Taxus). In Cupressus the pollen-grains not unfrequently protrude tubes, whilst still in the pollen-sacs; in these free tubes a cell-formation by division frequently takes place.

3. The scale which carries the pollen-sacs of the above-named Coniferæ (which pollen-sacs have frequently been taken for the true anthers) is shown by following out the developement to be the true anther of these plants. The pollen-sacs are developed by a peculiar formation of leaf-tissue at certain parts of these anthers, such part in Thuja, Cupressus, Callitris, and Juniperus communis, being the underside of the stalk. In Taxus, on the other hand, the pollen-sacs appear in the form of five or six loculi on the inner side around the stem of the shield-like anthers; they open like the others by a fissure. The pollen-sacs of the Cycadeæ also are certainly not to be regarded as real anthers, but as those parts of the anther which perfect the pollen; the scale which bears this pollen-sac is the true anther. The male blossom (the catkin) of the Coniferæ and Cycadeæ consists therefore of a stem-like portion, the axis, upon which a congeries of leaves are perfected as anthers; it is to be regarded as a single blossom, whilst the male catkins of the Amentaceæ really have distinct flowers.

4. In Podocarpus the construction of the anther is similar to that in the Abietinæ; it is bilocular; the loculi are situated

towards the outer lower side; they dehisce like the anthers of Pinus and Larix, by a perpendicular longitudinal fissure. The pollen has an excrescence on either side, exactly as in Abies, Picea, and Pinus. The fissure in the cuticle intended for the exit of the pollen-tube lies between the two lateral excrescences. Ephedra, which has eight (? always) bilocular anthers seated upon a short column, has oval pollen-grains with six longitudinal ribs, upon which no place of egress for the pollen-tube is is to be discovered. The male blossoms of Podocarpus and Ephedra which I examined were dried specimens.

5. The pollen of the Corniferæ and Cycadeæ, if once dried, never becomes sufficiently saturated to enable the observer to come to any conclusion as to the division of its pollen-cell. This investigation, therefore, can only be undertaken with fresh pollen. It is then seen that the Cycadeæ of our hot-houses frequently do not perfect their pollen. In the botanical garden of Berlin, the pollen of a Zamia was found to be imperfect, that is, its contents were shrivelled up. In Juniperus communis, where the pollen-grain lies for a year apparently inactive upon the nucleus of the ovule, the division of the pollen-cell seems to take place for the first time in the second Spring; even in Taxus, where the pollen-grain lies inactive upon the nucleus for some weeks, the division of the pollen-cell not unfrequently takes place within the anthers; in Thuja and Cupressus the division may be seen even before the shedding of the pollen. The same thing occurs in Larix, Abies, Picea, and even in Pinus, although the pollen-tubes of the latter do not reach the embryo-sac and corpuscula until the following year.

6. As the cuticle of the Coniferæ is stripped off when the pollen-tube is developed, the termination of the latter, when it has reached the nucleus in Abies, Picea, Pinus, and Larix, is formed of the pedicel-cells of the cellular body, upon which the remnants of the lacerated covering of the true pollen-cell, and of the larger derivative-cell which originated in the pollen-cell, are often found hanging. In Cupressus, Thuja, Juniperus, and Taxus, on the other hand, the small undeveloped derivative-cell forms the termination of the pollen-tube, and upon this derivative-cell the remains of the broken membrane of the pollen-cell

may often be seen. The detached collapsed cuticle may not unfrequently be seen upon the nucleus near the pollen-tube.

7. The contents of the pollen-grains in the Coniferæ vary considerably, according to their state of developement: large and small starch-grains are to be met with, as well as fine granular matter devoid of starch. In Cupressus, when the pollen has protruded tubes in the interior of the pollen-sac, large round starch-grains are often found in the pollen-tube, but never free cells. Cell-formation in the pollen-tube seems always to take place by division; every cell has a manifest nucleus.

As far, therefore, as my observations have extended, there would seem to be two modes of formation of pollen-tubes in the Coniferæ.

First.—The pollen-cell becomes divided into two unequal halves; the smaller derivative-cell is further developed, and forms by repeated cell-divisions a cellular body, the terminal cell of which, being nourished by the contents of the larger derivative-cell of the pollen-grain, becomes the pollen-tube; the pollen-tube throws off the cuticle, and breaks through both the membrane of the true pollen-cell and that of the larger derivative-cell. This mode of formation of the pollen-tube obtains in Abies, Picea, Pinus, Larix, and perhaps in Podocarpus.

Secondly.—The pollen-cell becomes divided in like manner into two unequal halves, but the smaller derivative cell does *not* become further developed; on the other hand, the larger derivative-cell forms the pollen-tube, which throws off the cuticle, and breaks through the membrane of the pollen-cell. This occurs in Thuja, Cupressus, Juniperus, and Taxus.

The wide difference which is thus found to exist between the pollen of different Coniferæ has given a new aspect to the question relative to the impregnation of these plants. A continued series of accurate observations is very much to be desired. My own are not yet by any means complete; but I may mention the following circumstances as deserving of particular attention.

In the first place, although Hofmeister* considers their existence probable, I have never found phytozoa in the pollen-

* *See* Hofmeister's "Keimung, &c., Höherer Kryptogamen," p. 132.

tubes of any of the Coniferæ which I have examined. The act of impregnation in the Coniferæ generally bears no resemblance to the formation (within the germ organ) of the embryo of the higher Cryptogamia.

Secondly.—Free cells never originate in the corpuscula of the Coniferæ ; what Hofmeister took for cells are round cavities filled with a clear fluid, and circumscribed by a finely-granular, more solid mass. They are the so-called *mock-cells* which occur not unfrequently elsewhere, even in the pollen-tube of one of the Conifers themselves, viz., Pinus sylvestris.

Thirdly.—Several pollen-tubes generally reach the embryo-sac through the tissue of the nucleus : I have counted six such tubes ; nevertheless, one pollen-tube can impregnate several corpuscula.

Fourthly.—The pollen-tube forces its way between the cells of the so-called Rosette ; that is to say, between those cells which cover the apex of the corpusculum : these latter cells are pushed far apart by the pollen-tube (in Pinus, for instance), which then appears like a bladder in the interior of the corpusculum : the *mock-cells* then make way for the pollen-tube in such a manner that they continue still visible in the lower part of the corpusculum : it seems generally as if the fluid contents of the latter cells became thinner upon the entry of the pollen-tube. From the above it seems clear that Hofmeister's opinion, according to which a cell existing in the corpusculum is impregnated by the pollen-tube, is founded in error, since no cell is present in the corpusculum before the entry of the pollen-tube. New and extended observations made upon Lathræa and Viscum have convinced me of the accuracy of Schleiden's theory, according to which the first cells of the future plant originate in the pollen-tube itself.

CHAPTER XI.

SOME FURTHER REMARKS UPON THE MODE OF IMPREGNATION IN PINUS SYLVESTRIS AND TAXUS BACCATA.

SHORTLY after the pollen-tube of Pinus sylvestris has penetrated into the corpusculum it becomes slightly swollen; it appears in the form of a small bladder in the apex of the corpusculum. A cell then originates in this bladder by the formation of a horizontal partition; this latter cell then swells into a round shape and becomes divided cross-wise into four derivative cells. The body formed by the four cells is now suspended by the short tubular fragment of the pollen-tube, and is shortly afterwards found at the bottom of the corpusculum, and therefore opposite to the place of entrance of the pollen-tube. This cellular body, out of which the so-called *lower rosette*, as well as the embryonal tubes, and the first cells of the embryo, are formed, penetrates quite gradually to the bottom of the corpusculum, becoming detached meanwhile from the tubular portion of the pollen-tube.

The fluid contents of the pollen-tube become (as has been mentioned above) clearer by degrees from above downwards, the lower denser part therefore, which is filled with mock-cells, supports the cellular body until it eventually reaches its destination and proceeds to develope itself further. Not unfrequently, however, a division of the four cells appears to take place in a horizontal direction at an earlier period, on which account the cellular body at the bottom of the corpusculum consists generally not of four simple rosette-like cells lying near one another, but of four double cells. I traced this cellular body through its formation out of the end of the inserted pollen-tube, as well as in its progress downwards; so that I have seen it at the apex of the corpusculum with its four cells fully developed, still united to the tubular portion of the pollen-tube. I have seen it after-

wards in the middle of the pollen-tube; and, lastly, I have seen it at its final resting place, at the bottom of the corpusculum. After the contemporaneous division of the four cells has taken place, and when therefore the body at the bottom of the corpusculum consists of eight cells, the four cells of the lower row become again divided in a horizontal direction; the body now consists therefore of four rows of cells lying over one another, each row containing four cells. The uppermost row of these cells undergoes no further development, their walls remain very delicate; the walls of the second row become much thickened, but the cells do not become further developed in any other way; this row forms the so-called *lower rosette*, to which are attached the rows of cells of the following, that is the third layer, out of which the embryonal tubes are formed. The rudiment of the embryo is developed from the bottom row of cells. By the tubular prolongation of the third row of cells the corpusculum is pierced through at the bottom, and the fourth row, forming the rudiment of the embryo, is carried downwards into the wedge-shaped loosened tissue in the axis of the albumen, in order to become developed into the embryo.

Although Schleiden and I both assumed that the pollen-tube of *all* the Coniferæ after entering the corpusculum became distended, and that it lay with its wall close to the wall of the corpusculum, nevertheless in Pinus this is certainly not the case. The pollen-tube remains at the apex of the corpusculum, it never becomes distended; if a careful section be made, the tubular end of it may be clearly seen in the form of a short stalk, when the cellular body has already reached the middle or even the bottom of the corpusculum; this short stalk answers to the Funiculus suspensorius of other phanerogams.

In the Yew (Taxus baccata) the pollen-tube lies like a bladder over the apex of the embryo-sac; it penetrates into the depressions under which the corpuscula are situated, and there is formed in its interior a body consisting of four cells, arranged like a rosette. This body is formed before the pollen-tube breaks through the loosened wall of the corpusculum, and it appears to originate by division, not by free cell formation. It is probably formed, like the similar four-celled body in the

pollen-tube of Pinus, by division of *one* mother-cell. I have frequently been fortunate enough, whilst the pollen-tube was lying over a corpusculum, to detach it in an uninjured state, so that I could fully and accurately examine the cellular body whilst within the pollen-tube. The apex, or rather the extension of the bladder-like pollen-tube in which the above-mentioned cellular body lies, penetrates into the corpusculum, and becoming distended, fills up by degrees the hollow of the latter. The cellular body then becomes enlarged by repeated, but not always regular, division, so that the number and arrangement of the cells is not always the same. The corpuscula of one and the same embryo-sac differ also amongst themselves in size and shape. I have succeeded in completely detaching, at different periods of developement, that portion of the pollen-tube which has penetrated into the corpusculum. Generally, however, the pollen-tube breaks off at the place where it passed the mouth of the corpusculum. The embryonal tubes are formed out of the upper layers of cells, which latter are formed within the corpusculum out of the cellular body existing in the pollen-tube before the entrance of the latter into the corpusculum. The rudiment of the embryo, on the other hand, originates from the lower layers of cells. By the growth lengthwise of the embryonal-tubes the rudiment of the embryo is (as in Pinus) carried downwards into the wedge-shaped loosened portion of the albumen which is situated beneath the corpuscula, and there proceeds to develope itself into the embryo. The corpuscula and the embryonal tubes dry up as soon as their office has been fulfilled; the same is the case in Pinus.

In Taxus the pollen-tube really becomes enlarged in the interior of the corpusculum, as Schleiden has already noticed; this, however, does not occur in Pinus. In Pinus the position of the corpuscula of the impregnated ovule is such that their apices turn upwards; the cone hangs downwards, so that the ovules have their micropyles directed upwards. In this case, therefore, the cellular body, or the four-celled embryonic vesicle, can sink downwards by gravitation; in Taxus, where the apex of the corpusculum of the impregnated ovule points downwards, the change of place of the embryonic vesicle could not be

brought about in the same way. The single ovule in Taxus hangs with its micropyle downwards. Here also the pollen-tube goes really to the bottom of the corpusculum. In Abies pectinata, where the cones are upright, the place of entrance of the pollen-tube into the corpusculum lies underneath, as in Taxus, so that the embryonic vesicle cannot reach the place of its further developement in the same manner as in Pinus. An examination of Abies pectinata is likely to afford interesting results, especially on account of the size of its corpuscula.

In Phormium tenax, Schleiden has pointed out the formation of the first cells of the embryo in the interior of the pollen-tube, whilst the latter was lying over the embryo-sac, and before the wall of the embryo-sac had been broken through. In Taxus I have been so fortunate as to establish indubitably the same result; viz., the formation of the first cells of the embryo within the pollen-tube whilst still lying over the corpusculum; thus affording direct evidence in favour of the correctness of Schleiden's theory in the case of the Coniferæ.

The idea, therefore, of the relation between the Coniferæ and the higher Cryptogamia, which was founded upon the mode of developement of the embryo, is no longer worthy of consideration. The Coniferæ, so far as regards their mode of impregnation, are united much more intimately with the rest of the phænogamous plants; they differ from the latter only in the circumstance, that their pollen-cell and embryo-sac are not directly, but to a certain extent indirectly, active in the formation of the embryo. Whilst, for example, in the other phanerogams the pollen-cell itself is prolonged, and forms the pollen-tube, in the Coniferæ the pollen-cell forms derivative-cells, one of which becomes the pollen-tube; moreover, the first developement of the young rudiment of the embryo takes place in the Coniferæ, not immediately in the embryo-sac, but in a certain derivative-cell of the latter; viz., in the corpusculum. With regard to all other circumstances, the other phanerogams exhibit corresponding analogies: the embryonal tubes, for instance, are similar to the long appendage by which the embryo of Tropæolum is suspended, which appendage consists of several rows of cells.

The rudiment of the embryo, therefore, in the Coniferæ also lies in the pollen-tube, in the interior of which the primary cells of the embryo originate: the contents of the corpusculum in the first instance afford nourishment to these cells, and at a later period the young embryo is fed in the usual manner by the albumen. The office of the pollen-tube of the Coniferæ, therefore, is not to impregnate, but to bear the germ: the embryo-sac of the plant is generally only the organ which nourishes the germ; it is analogous to the uterus, in which the ovum in the animal kingdom is perfected. The mode of impregnation of phanerogamous plants, including the Coniferæ, bears no resemblance to the mode of impregnation in the animal kingdom; and just as little can the formation of the fruit, or embryo of the higher Cryptogamia, be compared with the fructification of the higher plants: *phytozoa* and *pollen-tubes* are widely different things.

Although Hofmeister (Flora, No. 17, 1854) has quite lately pointed out the entrance of the phytozoa into the interior of the germ-organ of ferns, the manner in which these phytozoa operate upon the free cell which, according to Hofmeister, exists within the germ-organ, is by no means understood; we must wait until careful observation shall have removed the veil under which these processes at present lie concealed.

APPENDIX.

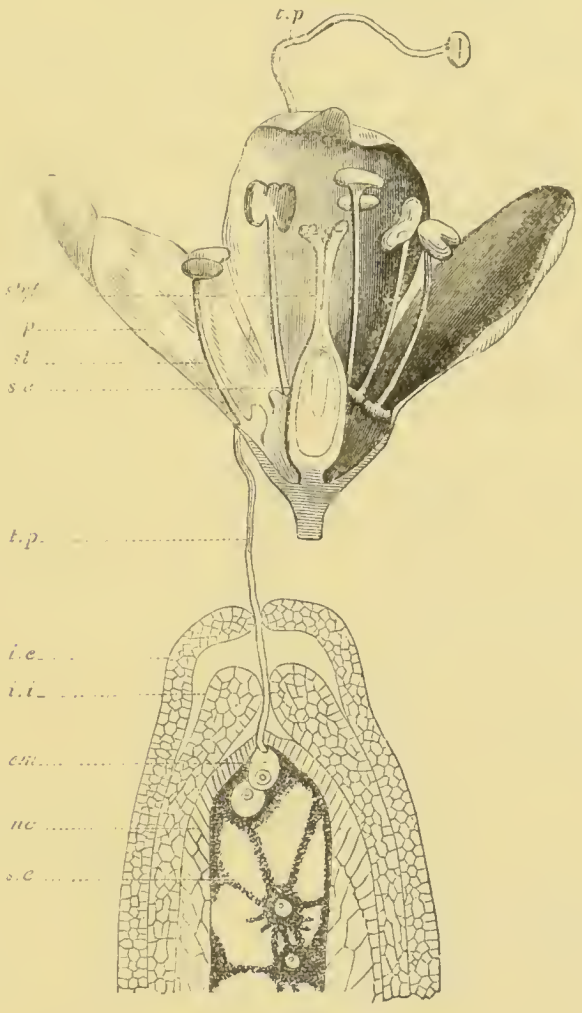
The following is Unger's account of the origin of the embryo, taken from the Twelfth Letter of his *Botanische Briefe*. After describing the formation of the ovule, he proceeds as follows :—

"Shortly afterwards, there is found in the centre of the ovule a cell, perhaps the top cell, which grows to a very large size; it is called the embryo-sac, and in its spacious interior several small free cells are produced, which float about in the contents of the former cell. These are, and can be, no other than reproductive cells. These reproductive cells do not appear of themselves to increase any further in growth, and they would wither and eventually decay if they were not moved to further developement by external agency. The same would be the case with the free pollen-grains. It comes to pass, however, that the free pollen-grains and the cells of the embryo-sac come together, and, whilst the former perish by the contact, there arises in the latter a capacity for further developement; the result of which is, that the whole ovule, now called *the seed*, becomes detached from the mother-plant, and the young plant, already formed, is placed in a condition to carry out, unaided, its own developement. This young plant naturally follows in every respect the type of the mother-plant. The reproductive cells of the ovule are undoubtedly enclosed within it, the ovule itself being generally situated in the hollow of the united carpels, that is, hidden in the ovary; but this does not prevent the free pollen-grains from coming into immediate contact with the reproductive cells of the ovule. This takes place in the following manner: the position of the ovary is such, that amongst the many thousand pollen-grains which become free after the dehiscence of the anther, some must necessarily come

in contact with it, and especially with its apex. This apex, called the style, which originates in the growing together of the apices of the carpels, and which, according to the form of the latter, is shorter or more elongated, spreads out somewhat more widely at the point called the stigma. Those pollen-grains which fall upon the stigma are acted upon by a moist secretion, continually exuding from the stigma, which super-induces a further development, a further growth, or, as one may say, a *germination*, the result of which is, that a cellular utricle is developed behind the external covering of the pollen-grain, which utricle, although single, is in a condition to branch out, by protruding itself through the external covering. The germinating pollen-grain, however, notwithstanding the moisture of the stigma, would soon perish, if it were not in a condition, by means of its projecting end, to make a passage for itself between the loose cells of the stigma, and between the slightly-connected tissue of the style. After some time one or more of the pollen-grains always succeeds in effecting a passage downwards into the ovary. There are now but few more difficulties to be overcome. The apex of the growing pollen-tube easily reaches the ovule, and finds there, through the openings in the coats of the ovule, an uninterrupted passage to the nucleus. Finally, however, the cells of the nucleus must be broken through. This is easily done, inasmuch as these cells are still very tender and yielding, and, at the same time, the embryo-sac, through its own extension and the displacement of the cells above, is, to a certain extent, brought in apposition to the pollen-tube. The germ-cells in the interior of the embryo-sac itself are found at this period near the surface; they even touch the inner side of its wall. It is, therefore, an easy matter for the pollen-tube, having penetrated thus far, to come into immediate contact with the germ-cells, from which it is only separated by the membrane of the embryo-sac; the pollen-tube even spreads itself over the surface of the embryo-sac, in order, if possible, forcibly to bring about this contact. The result is, that whilst the pollen-tube withers by degrees, the process of decay being from the exterior inwards, a further cell-formation commences in one of the germ-cells, probably in that one which

lies next to the pollen-tube, which cell-formation terminates in the production of the rudiments of a new plant. It appears not improbable that in many cases the embryo-sac becomes completely absorbed at the point of contact of the pollen-tube, so as to enable the pollen-tube and germ-cells to come into immediate contact with one another; but this is not yet sufficiently proved by experience."

The annexed figure is given by Unger in illustration of the above views. It represents a magnified longitudinal section of



the flower of Fagopyrum emarginatum. (*p.*) is the perianth; (*st.*) the stamens, the anthers having already burst. Some of

the pollen-grains are shown in contact with the stigma. They have already become elongated, and have penetrated through the canal of the style down to the embryo-sac. By magnifying the upper part of the ovule more highly, as in the lower figure, the whole course of the pollen-tube (*t.p.*) may be traced successively through the micropyle, the integumentum externum (*i.e.*), the integumentum internum (*i.i.*), and the nucleus (*n.c.*), down to the embryo-sac (*s.c.*), where it comes into immediate contact with the germ-cells (*e.m.*)

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THE END.

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